# Aging & Rehabilitation

## An Interdisciplinary Research Seminar Series


# Sponsors

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- Rehabilitation Outcomes Research Center (RORC)
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**UF College of Nursing**

# Schedule

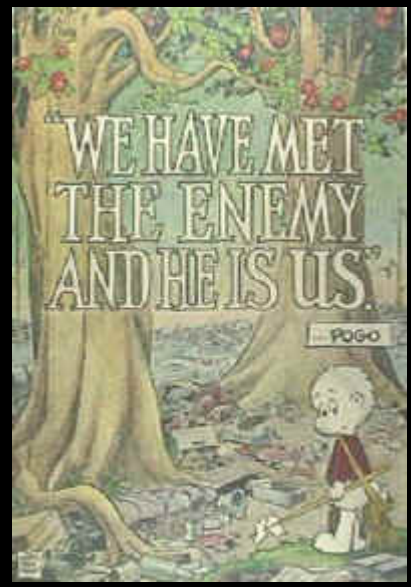
- January 9th, 2006 – May 22nd, 2006
- Mondays, 12:00 – 1:00
- Location: UF HPNP Building, Room G101
- Cyber Seminar:
  - VA RORC Conference Room, Commerce Building Downtown
  - VA BRRC Nursing Home Care Unit Conference Room (first floor)
  - UF Brooks Center Conference Room, Jacksonville (904) 306-8977

# Themes

- Basic Science
- Clinical Science
- Outcomes / Health Policy
- Behavioral and Social Research
- Cutting Edge / New Research

# Inflammation, Atherosclerosis & Aging

"Yep, son, we have met the enemy and he is us!"
Pogo to Porky (as written by Walt Kelly), 1971




**Russell P. Tracy, Ph.D.**
**Professor of Pathology and Biochemistry**
**University of Vermont College of Medicine**

**http://www.med.uvm.edu/lcbr**
**russell.tracy@uvm.edu**

# Laboratory for Clinical Biochemistry Research at the Colchester Research Facility

## Current snapshot 1/2006

### Investigators

| | |
|---|---|
| Russ Tracy, PhD | *Prof Pathol, Biochem* |
| Ted Bovill, MD | *Prof, Chair Pathol* |
| Sally Huber, PhD | *Prof Pathol* |
| Mary Cushman, MD, MS | *Assoc Prof Med, Pathol* |
| Nancy Jenny, PhD | *Res Asst Prof Pathol* |
| Peggy Doyle, PhD | *Res Assoc Pathol* |
| Michael Lewis, MD | *Asst Prof Pathol* |

### Post-Doctoral Fellows/Associated Scientists

Dan Jones, MD
Jan Carney, MD
Dom Geffken, MD, MPH

### Graduate Students

Nels Olson

### Unit Administrator

Kevin Kolinich

### Supervisory Staff

| | |
|---|---|
| Elaine Cornell | *Lab Coordinator* |
| Rebekah Boyle | *Asst Lab Coordinator* |
| Peter Durda | *DNA Lab* |
| Liz Macy | *Assay Development* |
| Bruce Scott, PhD | *Linkage studies* |
| Danielle Sartini | *Animal models* |

### Technical Staff

Dean Draayer, PhD
Kate Durda
Kanene Felo
Nicole Gagne
Christine Germano
Florence Keating
Vicci Letourneau
Laura Lynch
Mohamad Moussawi
Sarah Nightingale
Angela Patnoad
Danielle Parent
Jill Perrotte
April Perry
Vanitha Rajendran
Brian Roberts
Nora Sullivan
Cathy Tilley
Julia Valliere
Mary Ellen Walker

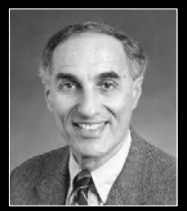
- Population-based studies and Family Studies: phenotypes, genotypes and haplotypes
- Clinical Trials: HRT, anticoagulation, exercise, diet
- Animal Models: murine atherosclerosis

# Atherosclerosis & Inflammation: the Beginnings…

Recently, low-level, chronic inflammation has been linked to atherosclerosis in clinical syndromes and then in the general population. However, the association of inflammation with atherosclerosis is not a new story….

"…inflammation of the inner arterial coat [is] the starting point of the so-called atheromatous degeneration."

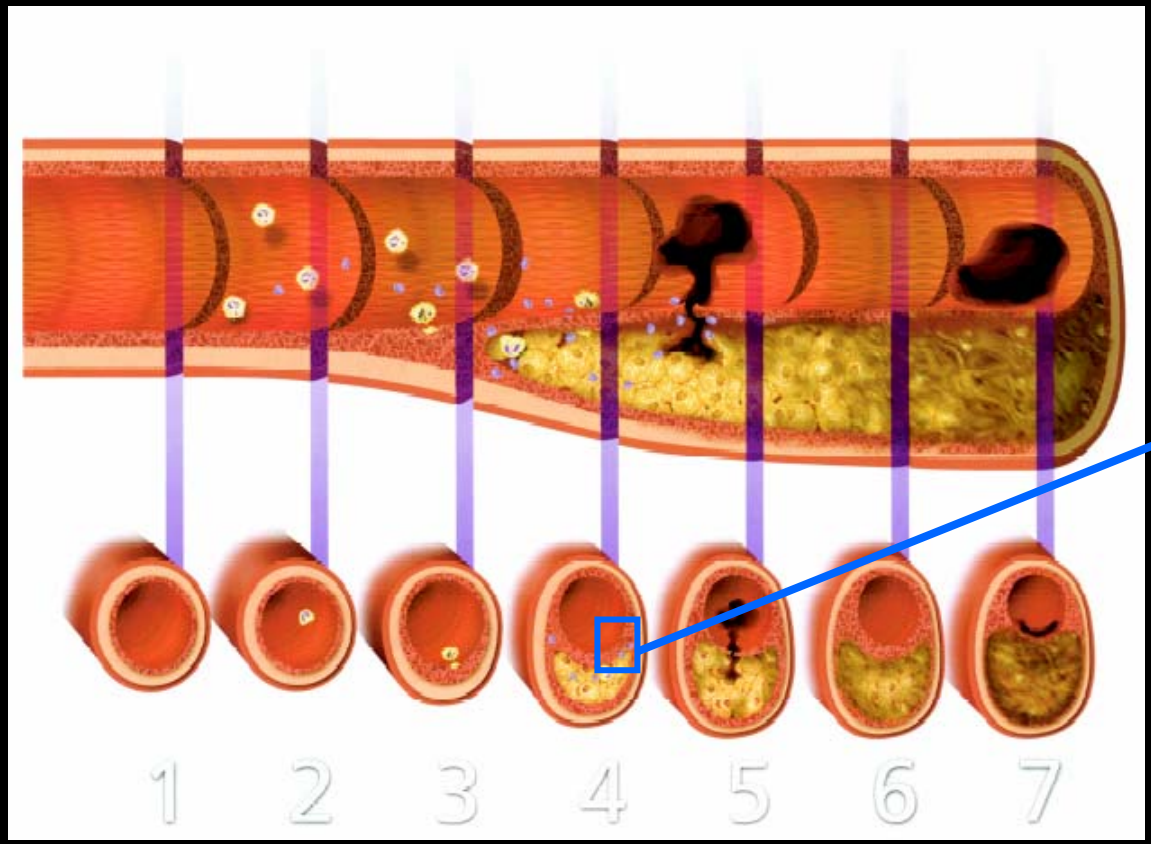
R. Virchow: *Cellular Pathology As Based Upon Physiological And Pathological Histology*. **1859**. English translation of a second German edition, Philadelphia PA, JB Lippincott, 1971, p.396 (as reviewed by Nieto, Am J Epidemiol, 148:937, 1998)



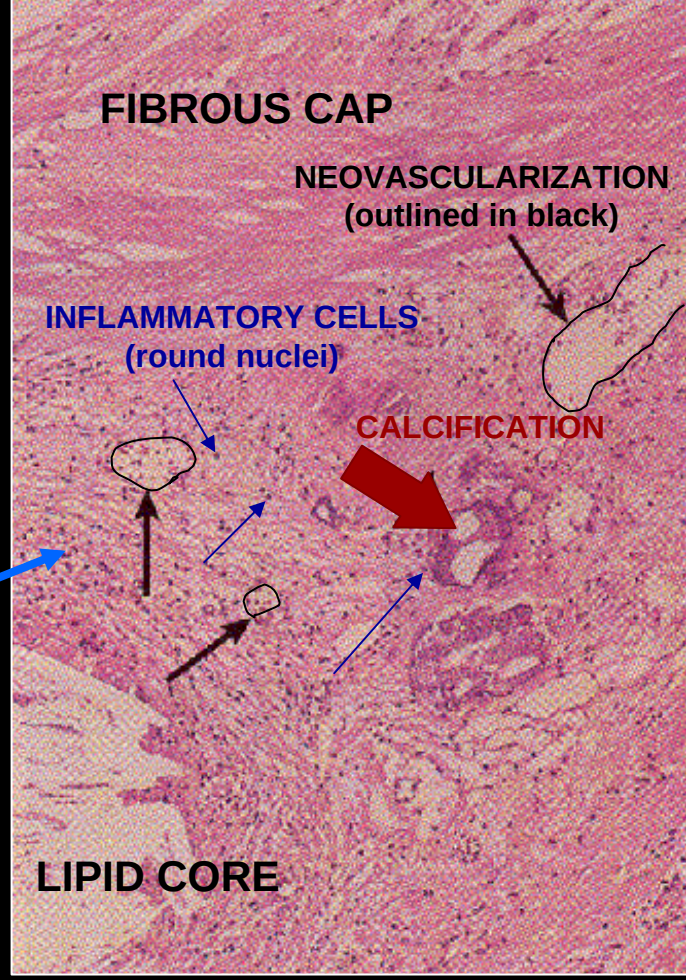
Atherosclerosis is a "…response to injury…"

Ross R, Glomset JA. The pathogenesis of atherosclerosis (first of two parts). *N Engl J Med*. **1976**;295:369-77

# Vascular Cell Biology




Libby P. *Circ* 104:365-72, 2001




Kotran, Kumar, Collins. *Robbins Pathologic Basis of Disease*. 6th edition. Saunders, 1999

**Atherosclerosis, thrombosis and calcification** → **Inflammation (Interleukin-6; IL-6)** → **C-reactive protein (CRP) Fibrinogen**

# Atherosclerosis

A model for Aging
And/or Chronic Diseases of Aging ?

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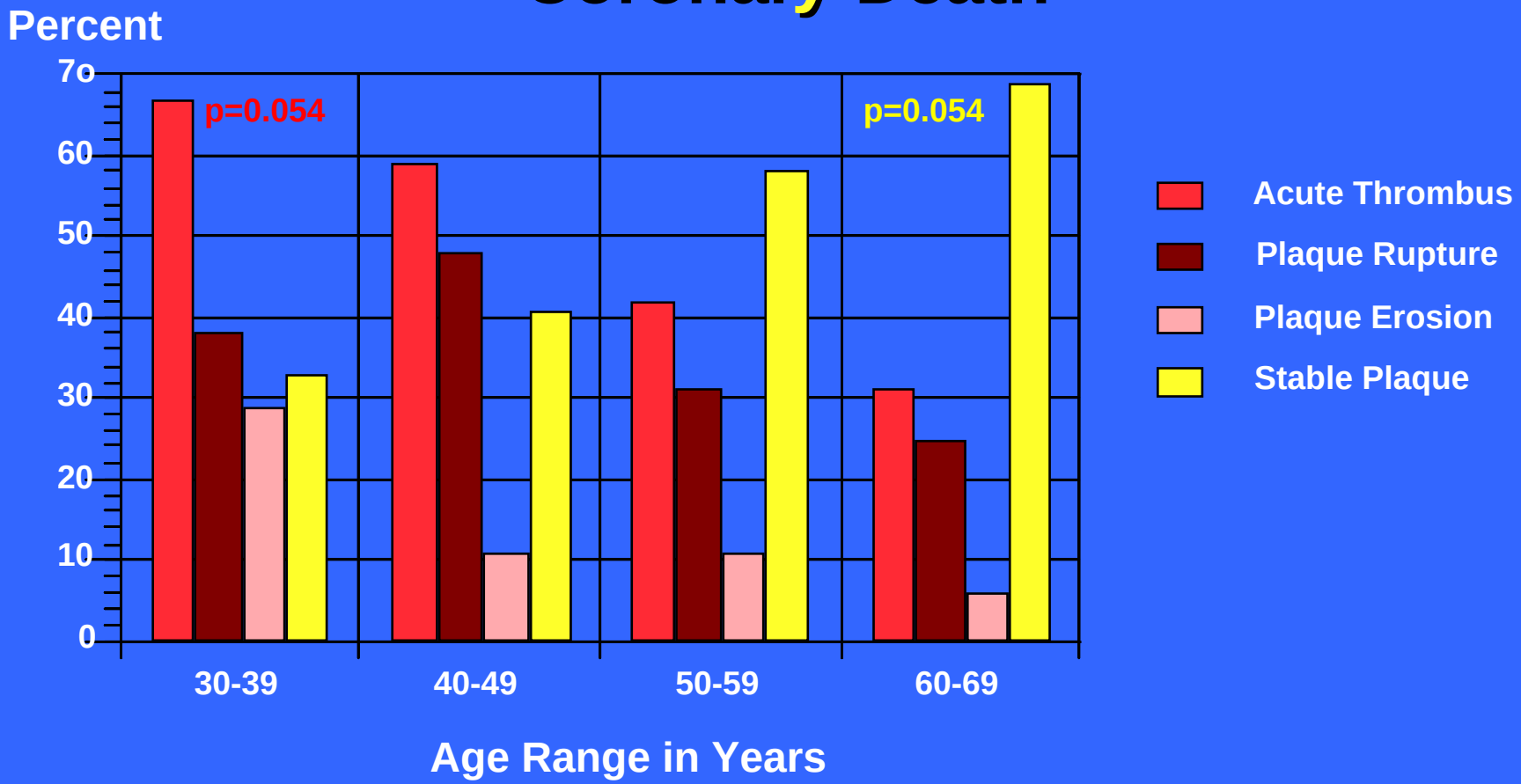
# Frequency of Coronary Thrombi in Culprit Lesions by Decade in Men Dying Sudden Coronary Death




R. Virmani, unpublished results, 2001

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How did we get here ??

A story of “atheroma” and “sclerosis”

One of today’s themes: does atheroma cause morbidity/mortality or does sclerosis…..

# Hypothesis: The Hypercoagulable State

- In the1970's DeWood and others: blood clots were the proximate cause of MI in many cases; was this a second dimension similar to lipids??

- This led to the hypothesis: a pre-existing "hypercoagulable state" predisposes to MI, much as had been shown for venous thrombosis

# Prothrombotic Factors

## Quick Summary

| | Thrombosis | | Assoc with |
|---|---|---|---|
| | Venous | Arterial | Inflammation |
| •Fibrinogen – | - | + | strong |
| •Factor VIIIc/vWF - | + | + | strong |
| •Markers of Process (e.g., D-Dimer) – | + | + | strong |
| •Factor Levels (e.g., inc FVIIc, dec PC) – | + | **(-)** | weak |
| •PAI-1 Levels - | - | +/- | weak |
| •Hypercoagulable genotypes – (e.g., FVLeiden) | + | **(-)** | none |

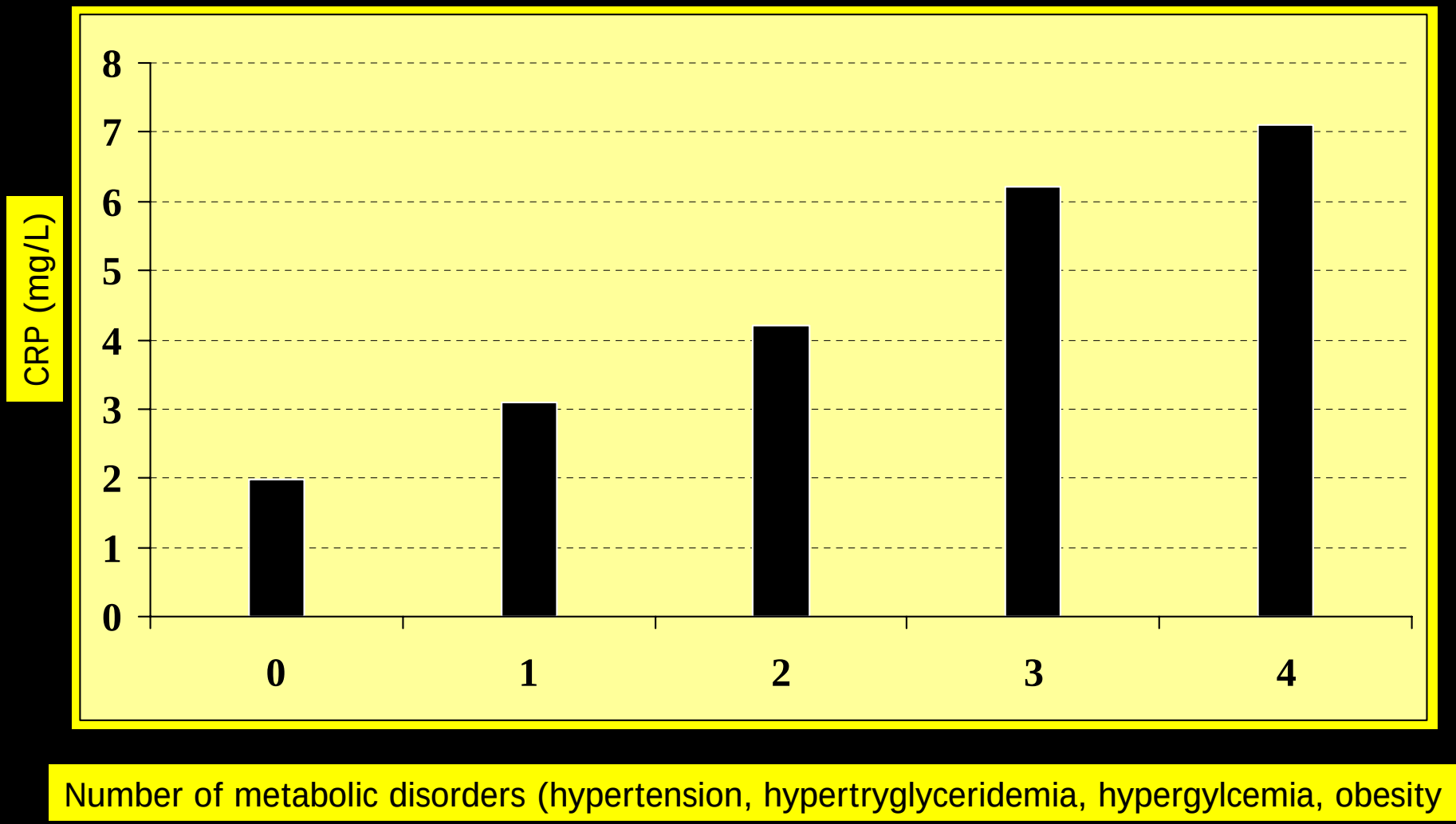
# Correlates of Inflammation

# Summary Analysis: Correlates of CRP

- Ethnicity (B > W)
- Gender (F > M)
- Age (+)
- Hypertension (+)
- Glucose tolerance status (++)
- Obesity (+++)
- HDL-C (--)
- Triglycerides (+)
- Insulin Sensitivity (++)
- Cigarette Smoking (+/-)
- Coag activity (++)
- IMT of the internal carotid artery (+/-)
- Coronary Calcification (-)

Howard G, et al. *Circulation*. 1999;99:1108; Festa A, et al., *Circulation* 2000;102:42-47; Tracy R, et al., *Arterioscler. Thromb. Vasc. Biol.* 1997;17:2167-2176; Folsom A, et al., *Am J Cardiol*. 2001;88:112-7; Redberg RF, et al. *J Am Coll Cardiol*. 2000;36:39-43

# Association of CRP with Components of the Metabolic Syndrome




Sakkinen P, Tracy RP. Unpublished results from the Cardiovascular Health Study

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# Biomarkers of Inflammation are Related to Vascular Disease

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# Most Recent Meta-Analysis of CRP and CVD Risk

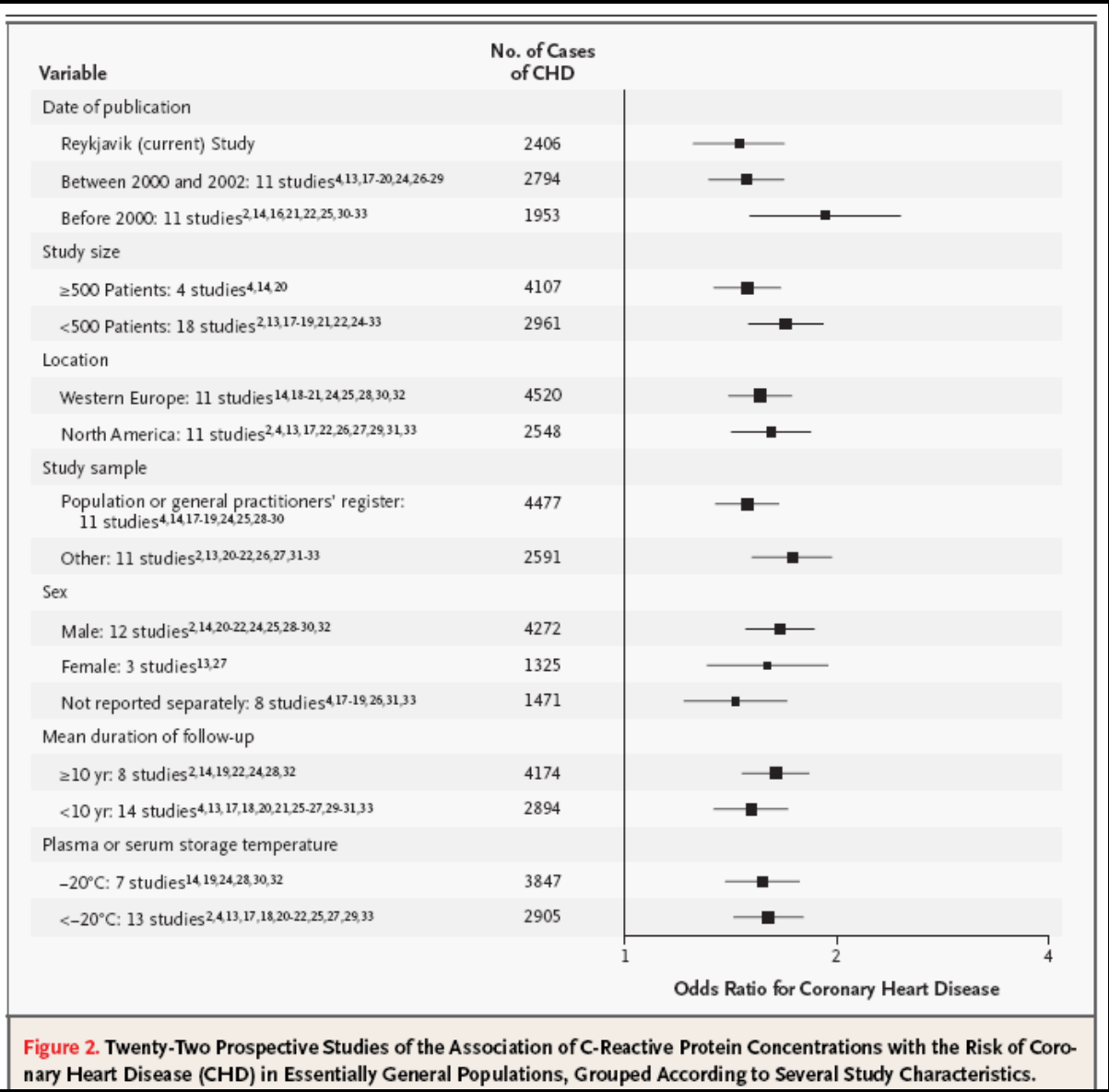


**Figure 2.** Twenty-Two Prospective Studies of the Association of C-Reactive Protein Concentrations with the Risk of Coronary Heart Disease (CHD) in Essentially General Populations, Grouped According to Several Study Characteristics.

## Danesh Meta-Analysis

- 22 studies
- men & women
- >7000 cases
- from 3 to 24 yrs followup

Danesh et al. *N Engl J Med* 2004;350:1387-97

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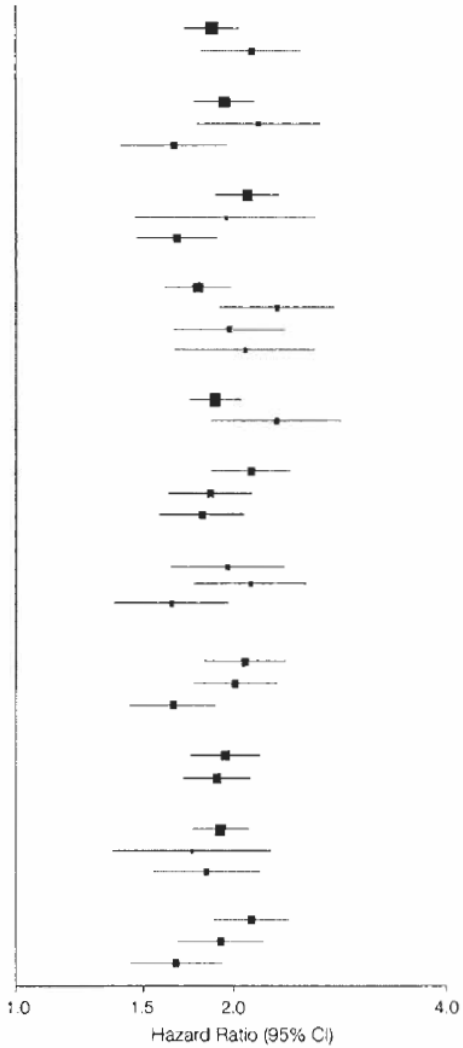
**Figure 4.** Adjusted Hazard Ratios for Coronary Heart Disease per 1-g/L Increase in Usual Fibrinogen Level

| Characteristic | No. of Coronary Heart Disease Cases |
|---|---|
| Geographic Location* | |
| Western Europe | 5369 |
| North America | 1718 |
| Study Population | |
| Population Registry | 4345 |
| Workforce | 974 |
| Prevention Trial | 1799 |
| Assay Method | |
| Clotting Time | 4382 |
| Clot Weight | 610 |
| Nonclotting | 2126 |
| Assay Timing† | |
| ≤A Few Days | 3611 |
| ≤A Few Weeks | 1252 |
| >A Few Weeks | 1224 |
| Not Stated | 1031 |
| Sex | |
| Male | 6171 |
| Female | 947 |
| Total Cholesterol, mmol/L (mg/dL) | |
| <5.73 (221) | 2376 |
| 5.73-6.73 (221-260) | 2378 |
| >6.73 (260) | 2364 |
| Triglycerides, mmol/L (mg/dL)‡ | |
| <1.29 (114) | 1175 |
| 1.29-2.00 (114-177) | 1165 |
| >2.00 (177) | 1145 |
| Systolic Blood Pressure, mm Hg | |
| <131 | 2450 |
| 131-150 | 2375 |
| >150 | 2293 |
| Smoking Status | |
| Former | 3784 |
| Current | 3334 |
| History of Diabetes | |
| No History | 4703 |
| History | 645 |
| Not Recorded | 1770 |
| Body Mass Index§ | |
| <25 | 2809 |
| 25-28 | 2300 |
| >28 | 2009 |




Adjusted for age at screening, smoking status, systolic blood pressure, total cholesterol, and body mass index and stratified by sex, cohort, and trial group. CI indicates confidence interval. The size of the data markers is proportional to the inverse of the variances of the hazard ratios.

*Osaka cohort has been excluded from geographical location.

†Relates to time following blood collection.

‡Tertiles of total cholesterol, triglycerides, systolic blood pressure, and body mass index were defined by their respective distributions among coronary heart disease cases.

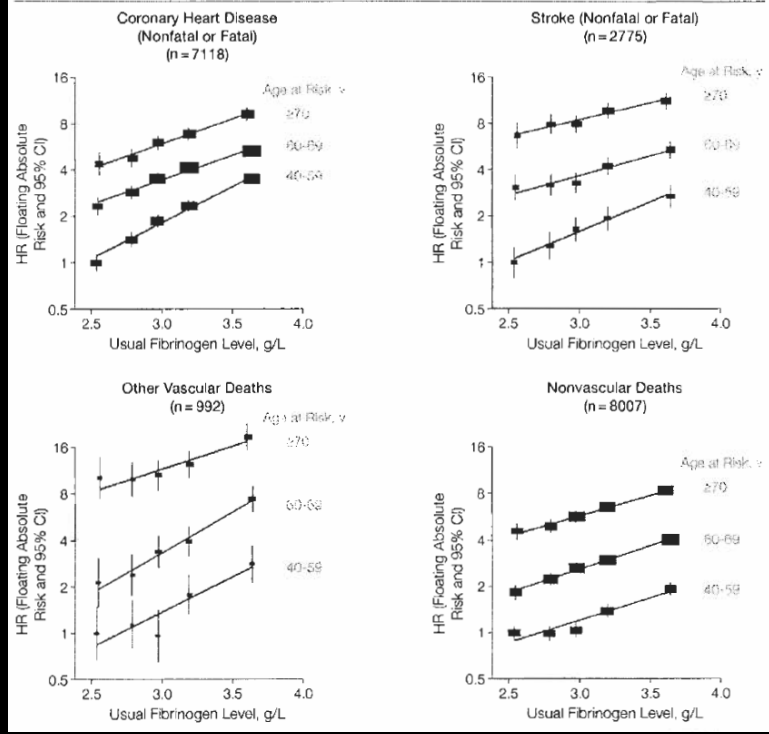
§Calculated as weight in kilograms divided by height in meters squared.

# Fibrinogen and CVD Risk:

Danesh J, et al. *JAMA* 2005; 294:1799-1809

- Danesh Meta-Analysis
- N = 154,211
- 31 prospective studies
- 6,944 MI cases
- 13,210 mortality cases

**Figure 1.** Age-Specific, Sex- and Cohort-Adjusted Hazard Ratios for Cardiovascular Disease and Nonvascular Mortality by Fifths of Usual Fibrinogen Level

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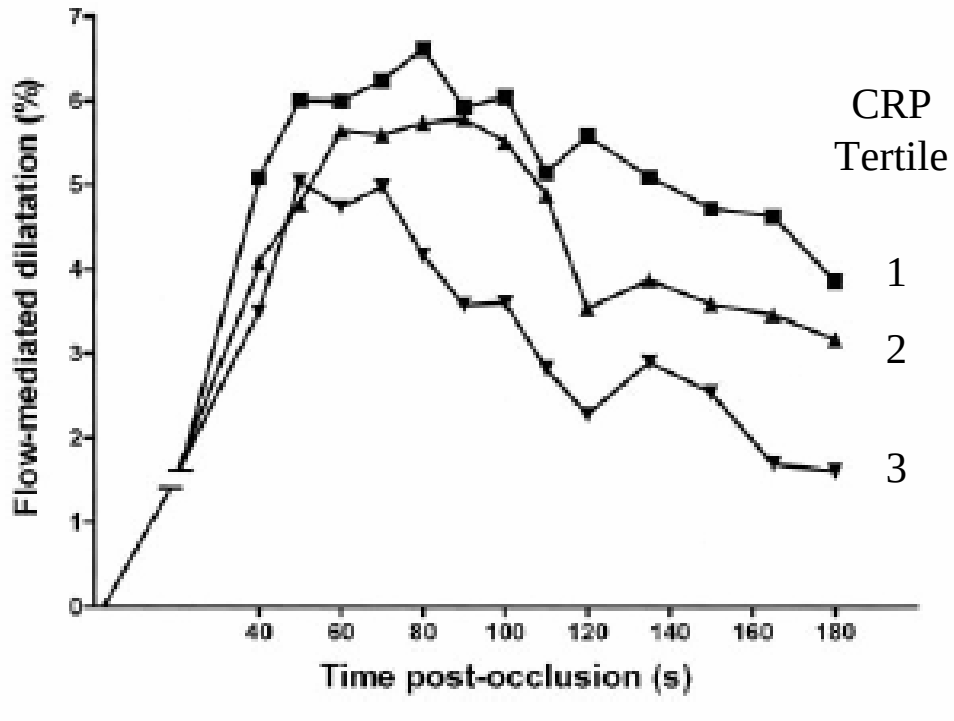
# Inflammation & Adiposity:

Unfortunately, adiposity and inflammation appear to intersect early in life….

# Adiposity-related proinflammatory changes in the young start at an early age

| | BOYS | | GIRLS | |
|---|---|---|---|---|
| | n | *r* | n | *r* |
| Age | 1479 | 0.13† | 1367 | 0.11† |
| BMI percentile | 1470 | 0.39† | 1358 | 0.41† |
| Systolic blood pressure* | 1093 | 0.20† | 1062* | 0.20† |
| Diastolic blood pressure* | 1093 | 0.09‡ | 1062* | 0.07 |
| Total cholesterol | 1455 | −0.01 | 1349 | 0.02 |
| Triglycerides | . . . | | . . . | |
| Glucose | . . . | | . . . | |
| HbA1c | . . . | | | |
| Homocysteine | 1478 | 0.04 | 1367 | 0.10† |

Correlation Coefficients Between LnCRP and CVD Risk Factors
Boys & Girls 3 to 17 Years of Age in NHANES 1999 to 2000




Flow-mediated brachial artery responsivity in 79 healthy boys and girls, mean age = 10.5 years

Ford ES. *Circulation*. 2003;108:1053-1058
Jarvisalo et al., *ATVB* 22:1323, 2002

# Adiposity, Sleep Disordered Breathing & Inflammation in Adolescents:

## Results from TeenZzz, a substudy of the Cleveland Children's Sleep & Health Study

**TABLE 4. Variation of CRP Levels With SDB**

| | Geometric Mean Values of CRP, mg/L* | | |
|---|---|---|---|
| | Unadjusted | Partially Adjusted† | Fully Adjusted‡ |
| AHI <1 | 0.42 (0.33–0.54) | 0.43 (0.33–0.56) | 0.50 (0.40–0.63) |
| AHI 1–4.9 | 0.56 (0.36–0.88) | 0.54 (0.34–0.86) | 0.43 (0.29–0.66) |
| AHI 5–14.9 | 1.48 (0.62–3.53) | 1.37 (0.56–3.34) | 0.97 (0.43–2.16) |
| AHI ≥15 | 3.11 (1.38–7.03) | 2.73 (1.17–6.37) | 1.66 (0.76–3.60) |

*Values are geometric means (95% confidence limits) of CRP (mg/L) values in unadjusted and adjusted models.

†Adjusted for age, sex, race.

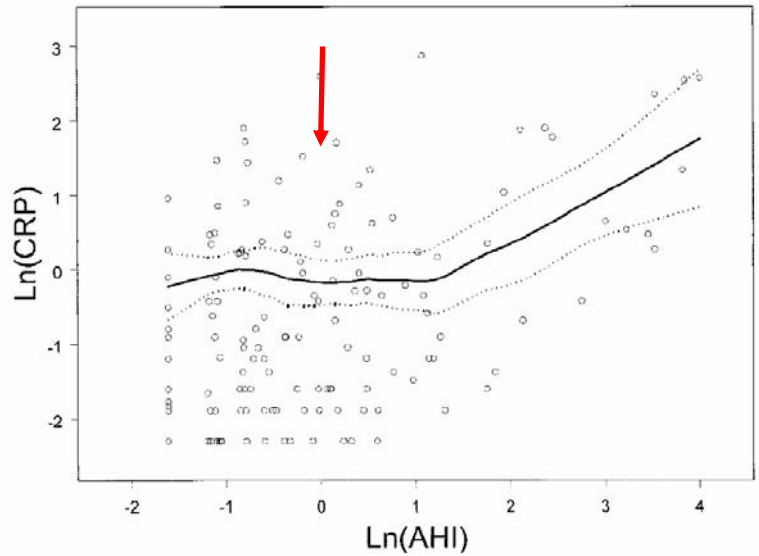
‡Adjusted for age, sex, race, BMI percentile, (BMI percentile$^2$).

**TABLE 5. Piecewise Multivariate Linear Regression Model Predicting (ln)CRP Levels**

| | β | SE | P |
|---|---|---|---|
| ln(AHI) | −0.0319 | 0.1232 | 0.796 |
| ln(AHI) ≥1.6* | 0.9129 | 0.3111 | 0.004 |
| BMI percentile | −0.0496 | 0.0173 | 0.005 |
| (BMI percentile$^2$) | 0.0006 | 0.0001 | <0.001 |
| Age | −0.0238 | 0.1154 | 0.837 |
| Male | −0.0834 | 0.2085 | 0.690 |
| Black | −0.1162 | 0.2156 | 0.591 |

*The slope of the line after ln(AHI) ≥1.6 is determined by summing the coefficients for ln(AHI) and ln(AHI) ≥1.6 (slope=0.8810; SE=0.2341; P=0.0002).

- N=143;
- age, 13 to 18 years;
- 36% black; 50% female;
- wide range of SDB quantified with the apnea hypopnea index (AHI) and oxygen desaturation measures.




Scatterplot of unadjusted ln(CRP) levels (based on average of 2 measurements) by level of ln(AHI), with line indicating mean adjusted ln(CRP) and pointwise 95% CI from the GAM.

Larkin et al., *Circulation*. 2005;111:1978-1984

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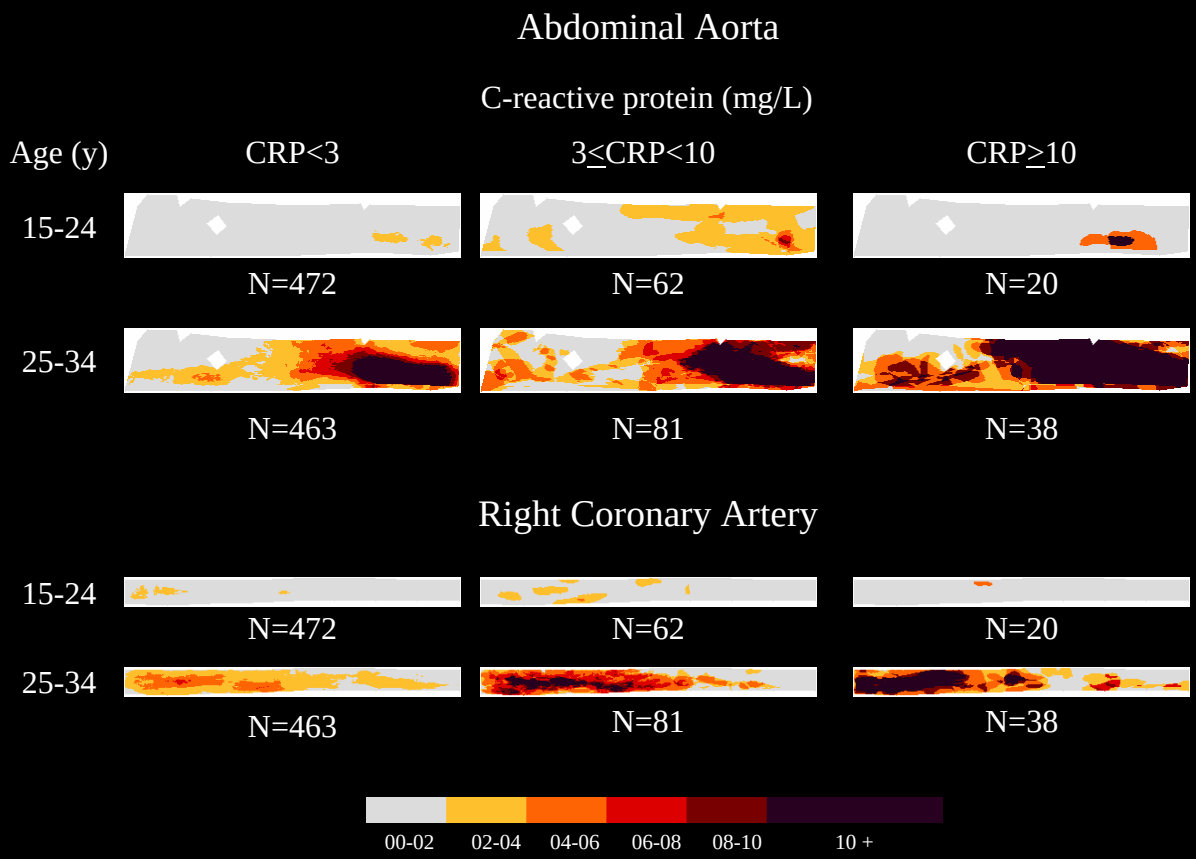
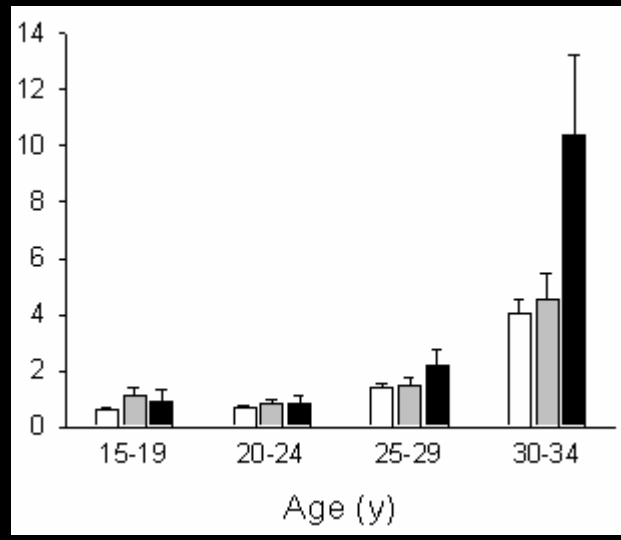
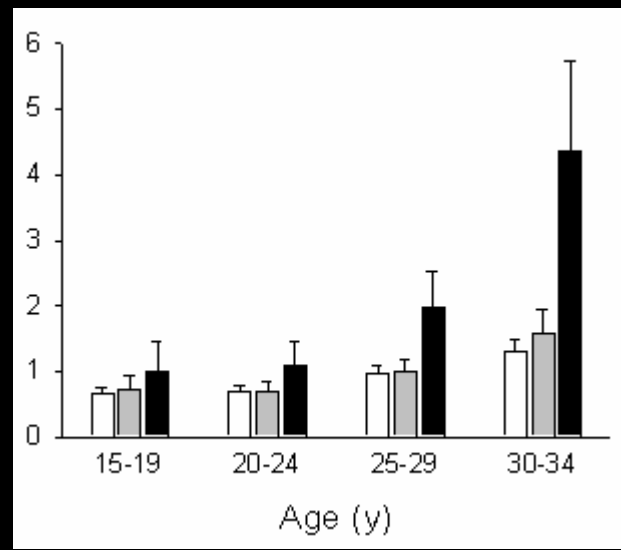
# Relationship of CRP and Atherosclerotic Lesions in Young Adults

## Results from PDAY (Pathobiological Determinants of Atherosclerosis in Youth)

N = 1136; 15 to 34 years; 50% Black, 28% women; died of accidents, homicides, and suicides; were autopsied within 48 hours after death




Zieske et al., *ATVB*, 2005;25:1237-1243

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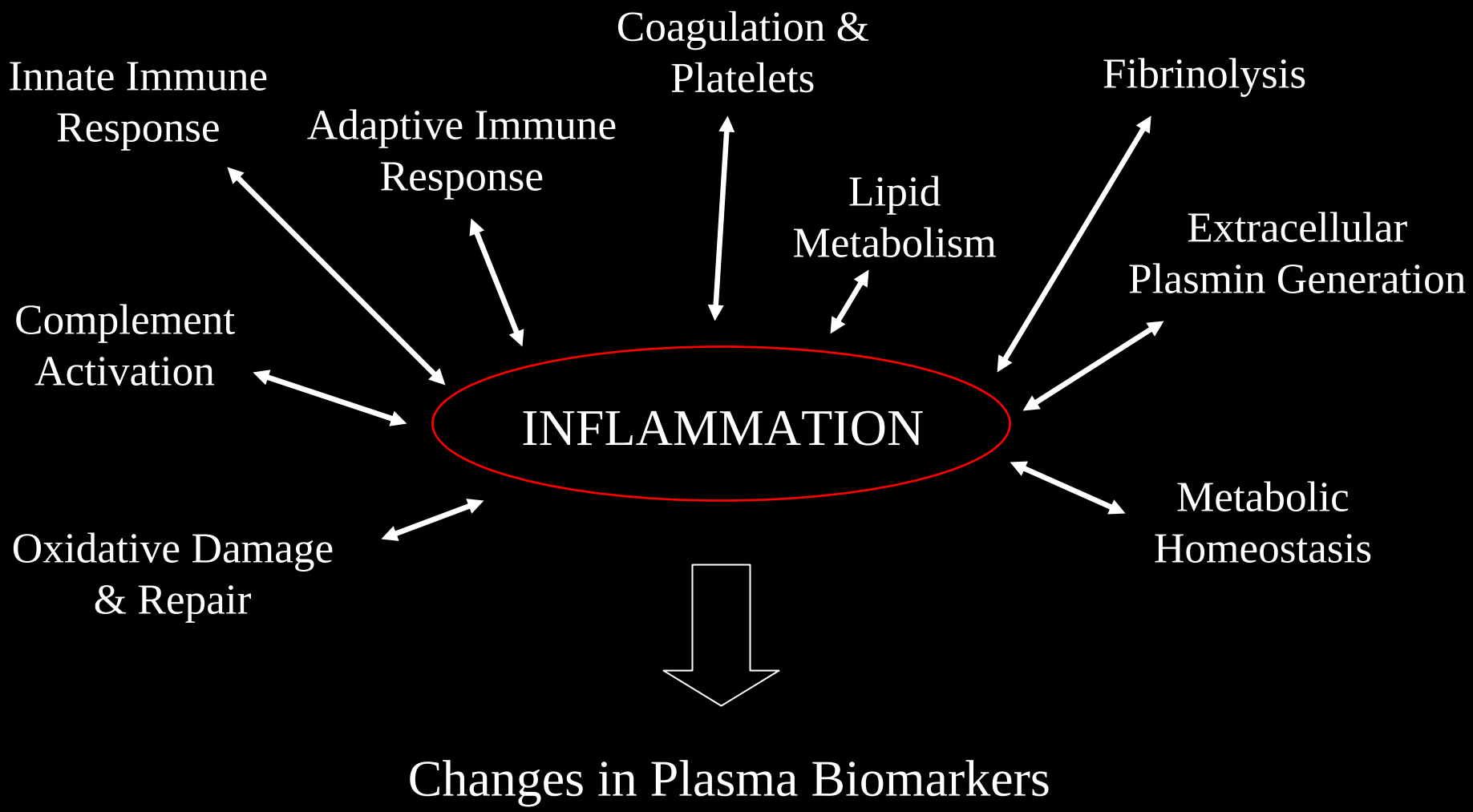
Many Markers of this Complex System show Relationships to Vascular Disease

And

These Markers are Related to Multiple Outcomes, not just Vascular Disease

# Cell Biological and Epidemiological studies have revealed many faces to “Inflammation”:



# Association of Markers of Inflammation With Chronic Disease of Old Age

## Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study

*Maria Inês Schmidt, Bruce B Duncan, A Richey Sharrett, Gunnar Lindberg, Peter J Savage, Steven Offenbacher, Maria Inês Azambuja, Russell P Tracy, Gerardo Heiss, for the ARIC Investigators*

| Marker | Model 1* (odds ratio [95% CI]) | Model 2 (odds ratio [95% CI])† | |
|---|---|---|---|
| | | All cases | First 3 years‡ |
| Sialic acid | 3·7 (1·4–9·8) | 2·8 (1·0–8·1) | 4·4 (1·1–16·8) |
| Orosomucoid | 7·9 (2·6–23·7) | 7·1 (2·1–23·7) | 7·9 (1·9–32·3) |
| α,-antitrypsin | 1·0 (0·4–2·4) | 1·1 (0·4–2·8) | 1·8 (0·6–4·9) |
| Haptoglobin | 1·7 (0·7–4·0) | 1·6 (0·6–4·1) | 2·1 (0·7–6·0) |

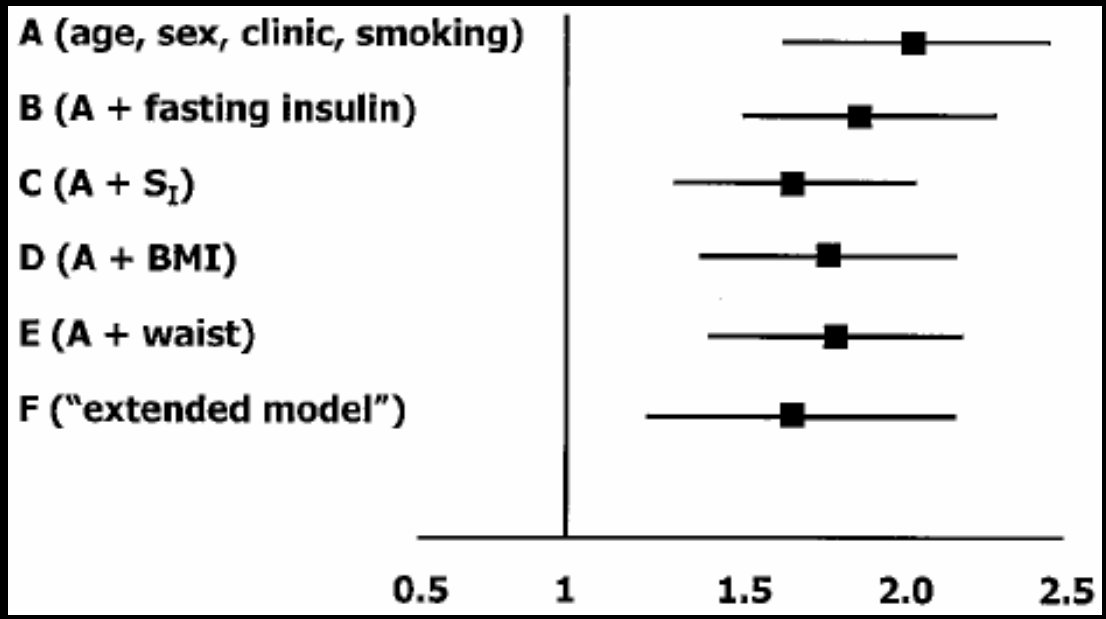
*Adjusted for age, sex, ethnic origin, atherosclerosis case-control status, fasting plasma glucose, family history of diabetes, and smoking status. †Adjusted additionally for body-mass index and waist-to-hip ratio. ‡Analysis only of diabetes detected at visit three.

Table 5: **Risk of developing diabetes mellitus for individuals in subgroup with values higher than the median for sialic acid and three acute-phase proteins**

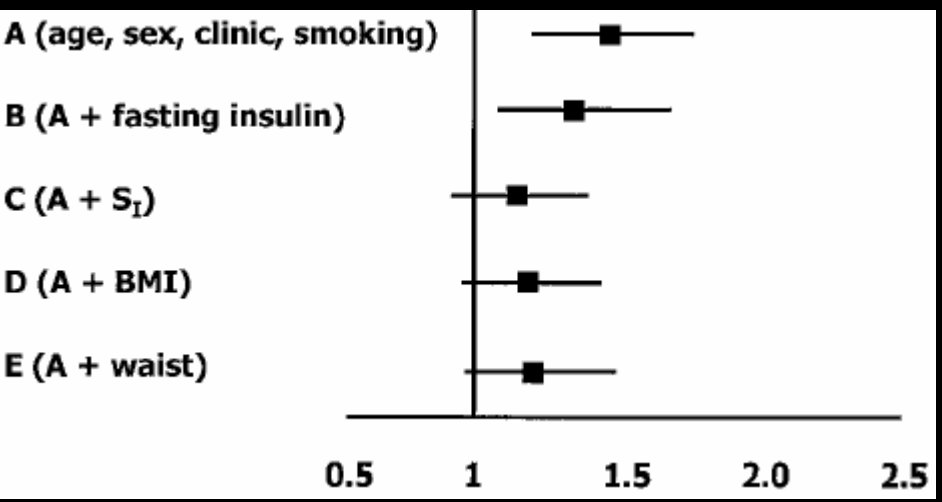
Schmidt et al., *Lancet*, 1999

# Association of CRP, Fibrinogen and PAI-1 with Risk of DM: IRAS

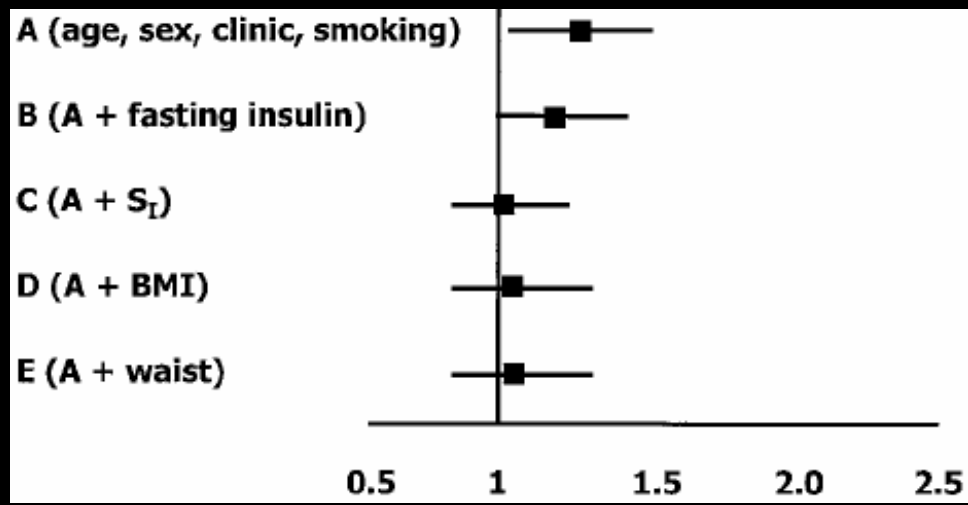
Model for PAI-1




Model for CRP




Model for Fibrinoger




Festa, D'Agostino, Tracy, Haffner.
*Diabetes* 51:1131-1137, 2002

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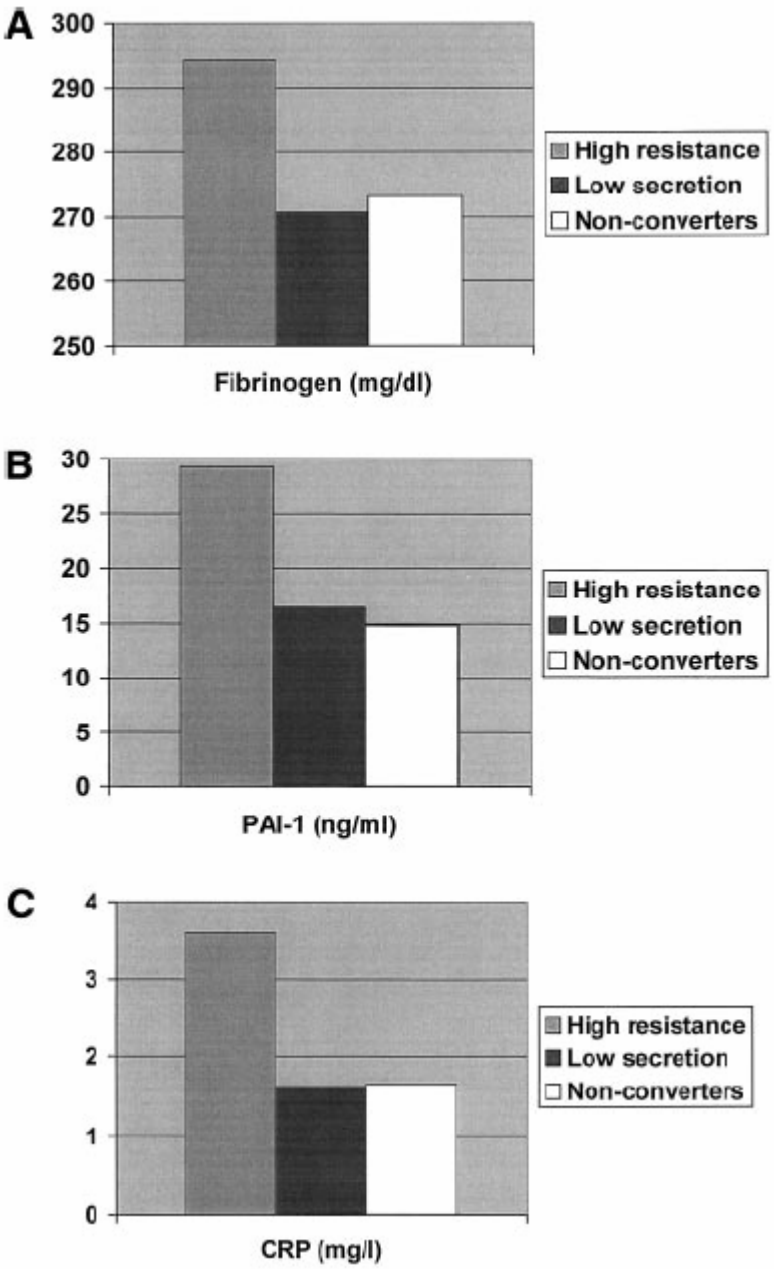


# Association of Inflammation Biomarkers with Insulin Resistance

In those who are destined to become diabetic (IRAS prediabetic subjects), biomarkers are associated with insulin resistance, not poor insulin secretion.

Festa A, Hanley AJ, Tracy RP, D'Agostino R, Jr., Haffner SM. *Circulation*. 2003;108:1822-30

# Association of Markers of Inflammation With Chronic Disease of Old Age

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PII S0735-1097(00)00582-9

**Heart Failure**

## Predictors of Congestive Heart Failure in the Elderly: The Cardiovascular Health Study

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Joseph F. Polak, MD,§ Russell P. Tracy, PHD,‖ Dalane W. Kitzman, MD, FACC,¶
Julius M. Gardin, MD, FACC,# John E. Rutledge, MD, FACC,** Robin C. Boineau, MD††

*Roslyn, New York; Washington, DC; Seattle, Washington; Worcester and Boston, Massachusetts; Colchester, Vermont; Winston-Salem, North Carolina; Irvine and Davis, California; and Bethesda, Maryland*

Gottdiener et al., *JACC*, 2000

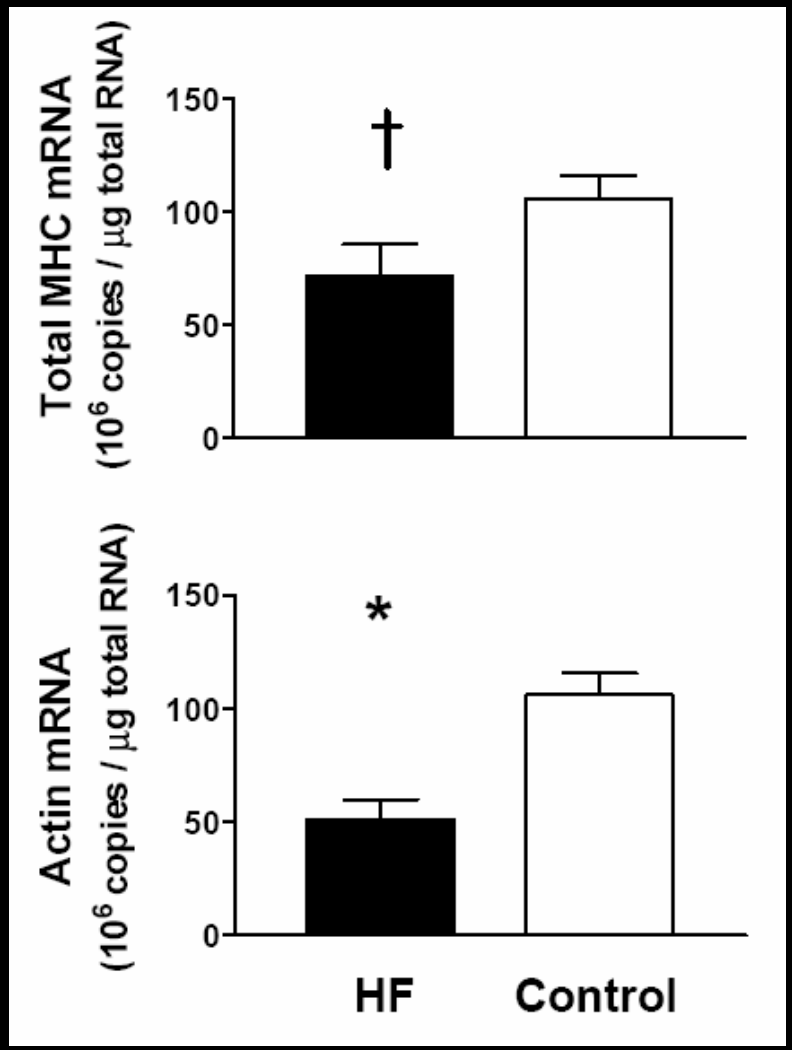
# Inflammation and Myofibrillar Protein Synthesis

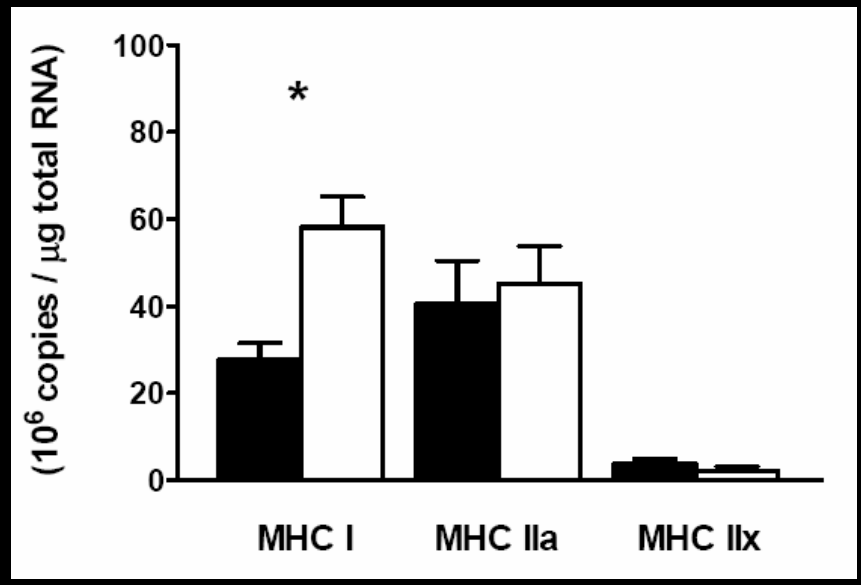
| | Heart Failure | Control |
|---|---|---|
| *n* | 9 | 9 |
| Age (yr) | 63 ± 4 | 70 ± 4 |
| Height (cm) | 176 ± 1 | 175 ± 3 |
| Body mass (kg) | 79 ± 5 | 80 ± 6 |
| Appendicular skeletal muscle mass (kg) | 26 ± 1 | 26 ± 1 |

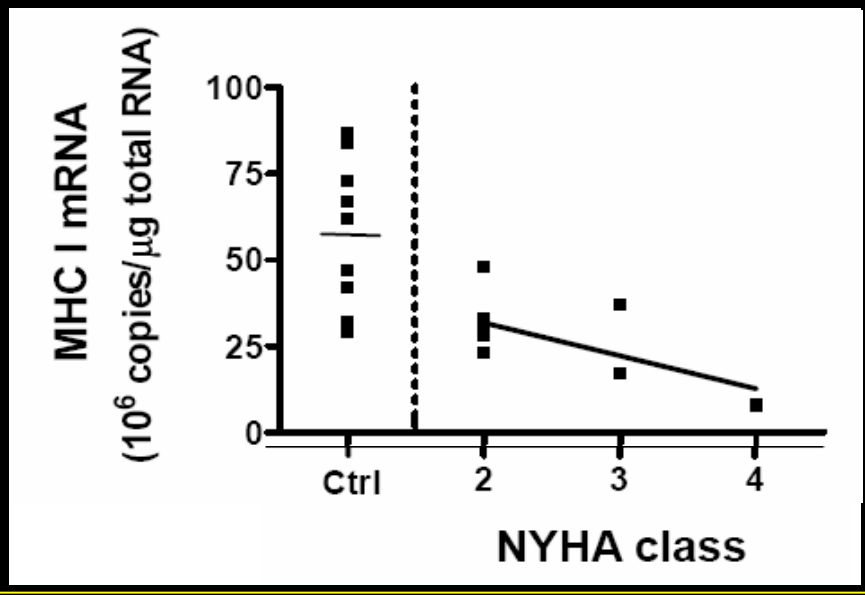
Data are mean ± SE.

Toth M, et al. *J App Physiol*, in press 2005

# Inflammation and Myofibrillar Protein Synthesis




Toth M, et al. *J App Physiol*, in press 2005

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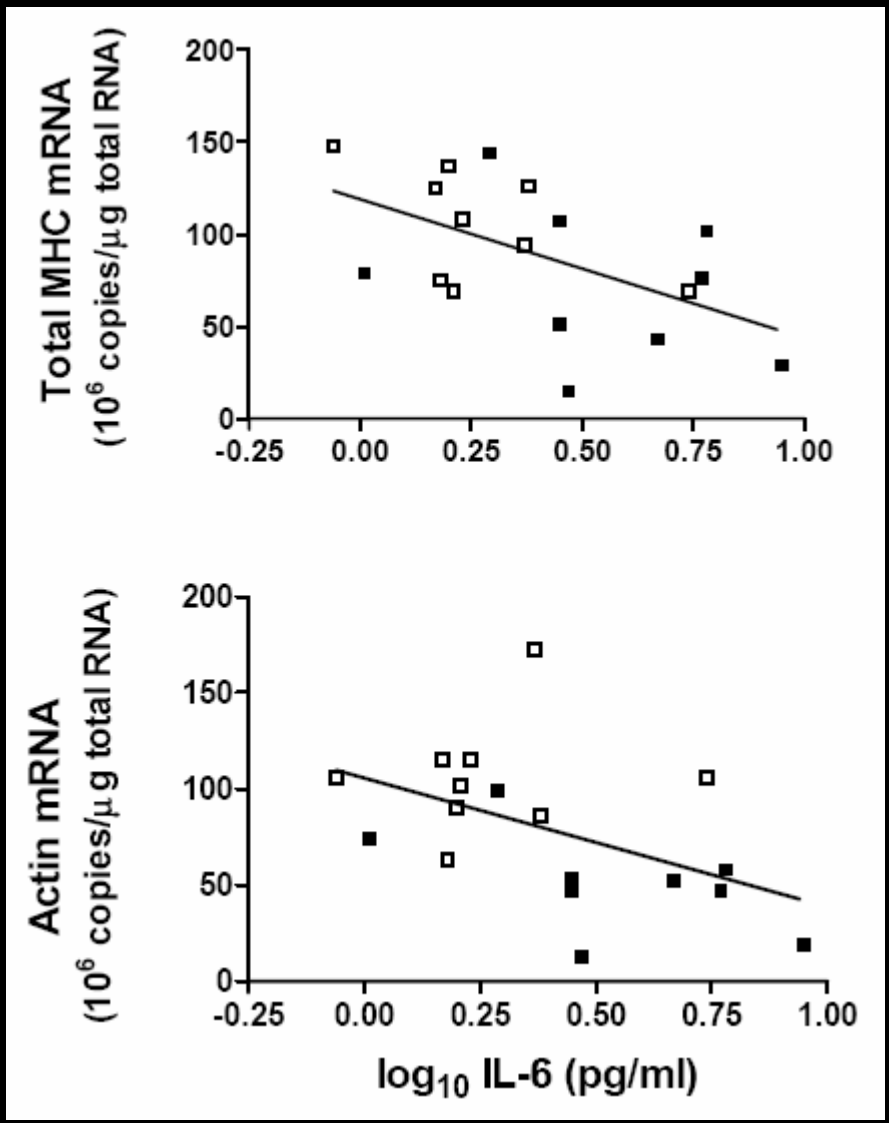
# Inflammation and Myofibrillar Protein Synthesis




"…. alterations in MHC protein content and isoform distribution in heart failure … [may be] … mediated by hormonal regulators acting via autocrine/paracrine and/or endocrine pathways …"

Toth M, et al. *J App Physiol*, in press 2005

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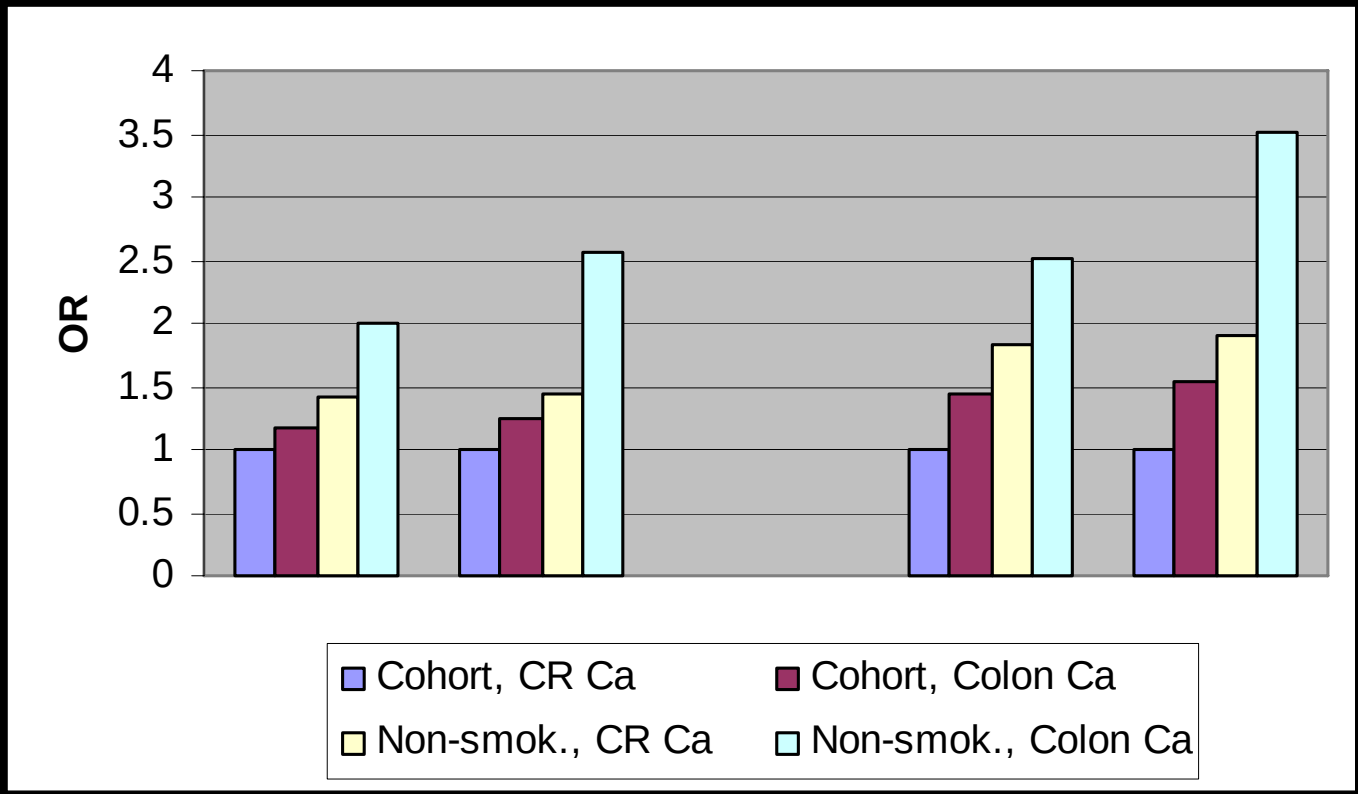
# CRP predicts future colorectal cancer




CLUE II

Prospective study of 22,887 men & women

11 years of follow-up, n = 172 cases

Case-Control design  2:1 matching on age, sex, race, date of blood draw

Erlinger et al., *JAMA* 291:585-590, 2004

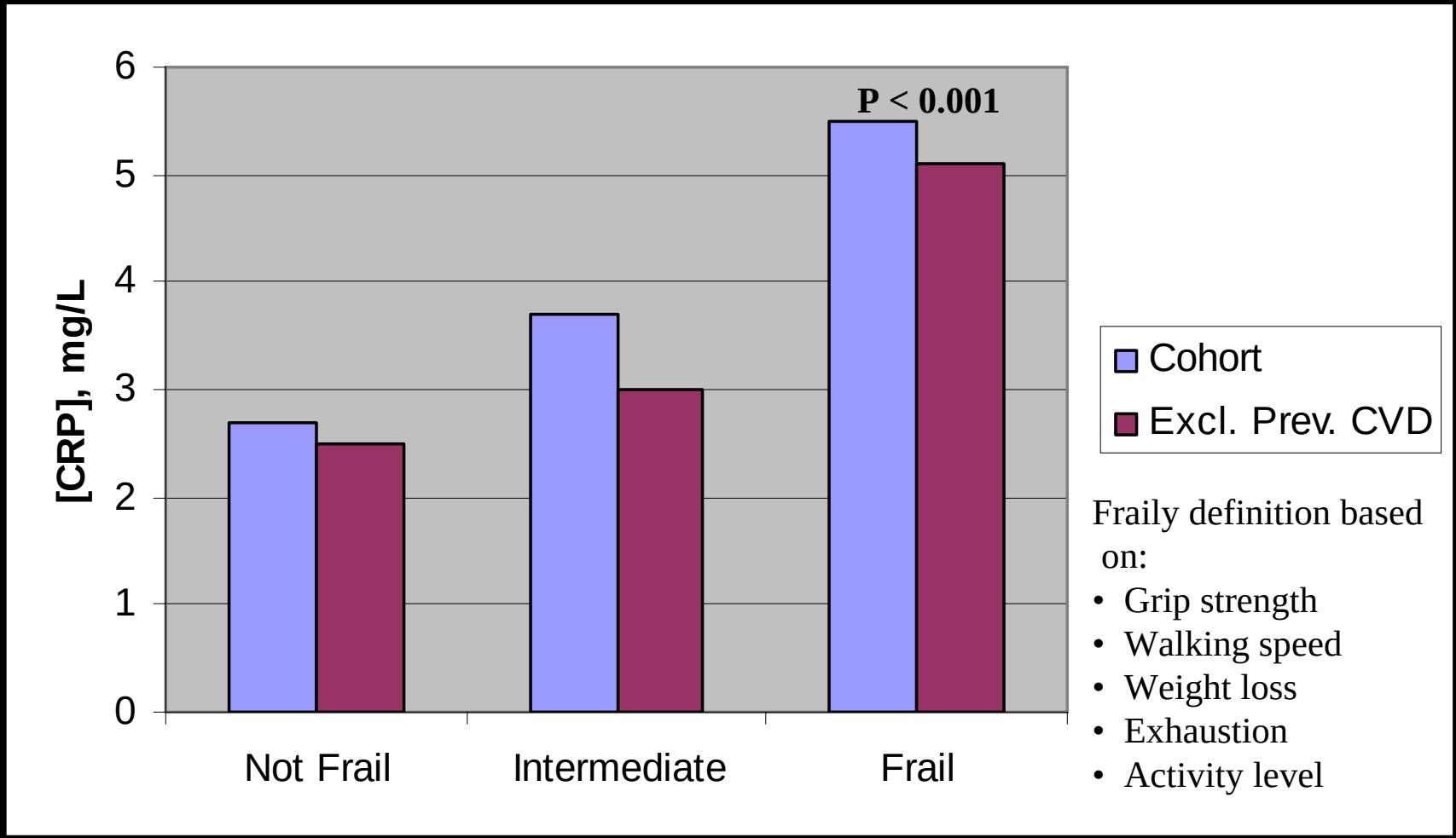
# Association of Markers of Inflammation With Chronic Disease of Old Age

- Japanese American men from the Honolulu Heart Program with 25 years follow-up for dementia in the Honolulu-Asia Aging Study
- Random subsample of 1,050 cases and noncases
- Measures
  - baseline C-reactive protein
  - dementia assessed by clinical exam plus neuroimaging and neuropsychological testing using international criteria

- Upper three quartiles for CRP vs first quartile: 3-fold increased risk for Alzheimer's disease or vascular dementia
- For vascular dementia alone, the risk increased with increasing quartile
- All results independent of cardiovascular risk factors and disease

Schmidt et al., *Ann Neurol*. 2002

# Relation of CRP to Frailty: CHS




Fraily definition based on:

- Grip strength
- Walking speed
- Weight loss
- Exhaustion
- Activity level

Walston et al., *Arch Int Med* 162:2333-2341, 2002

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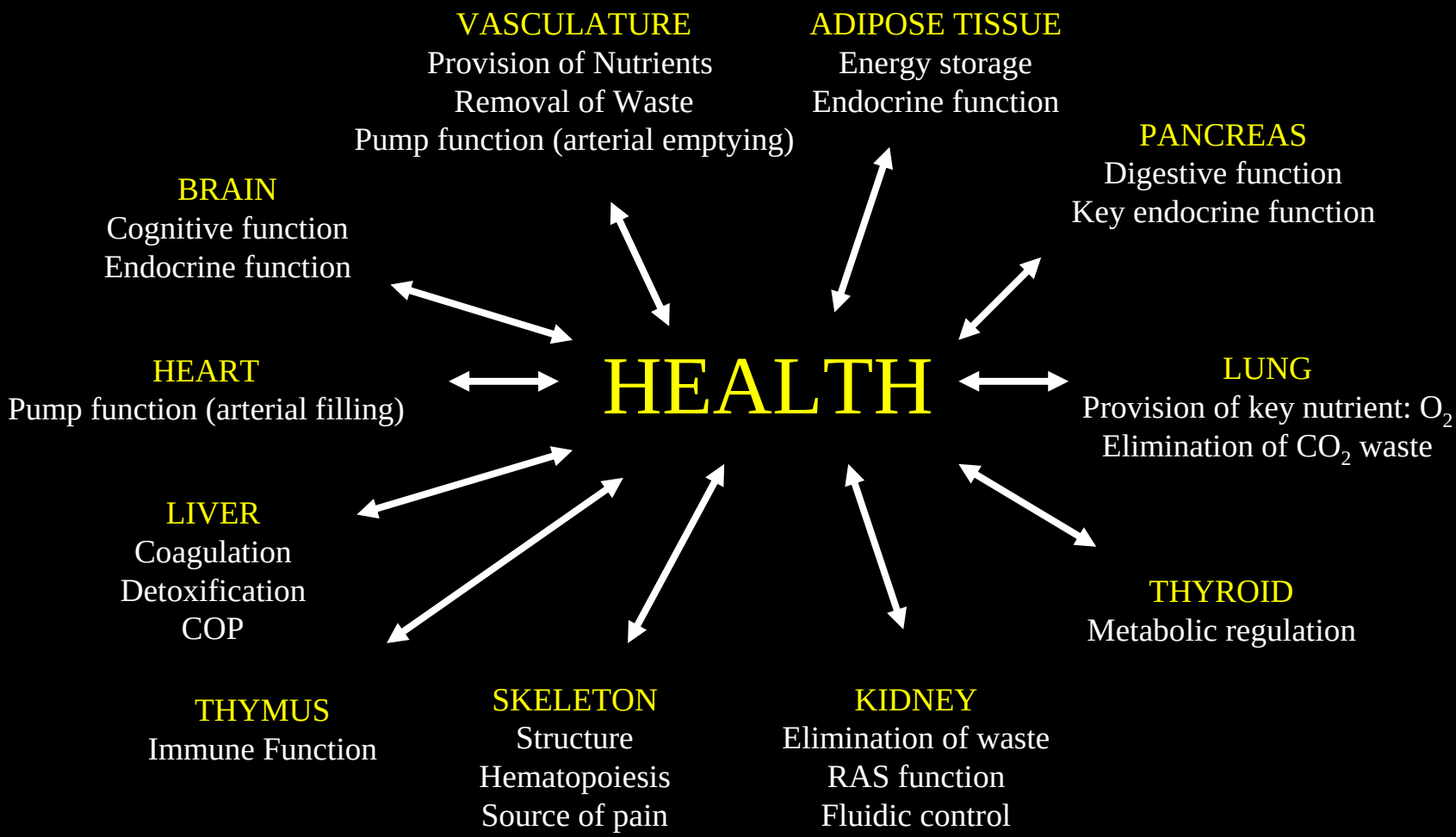
# Humans as integrated organisms: a decline in one system affects all??

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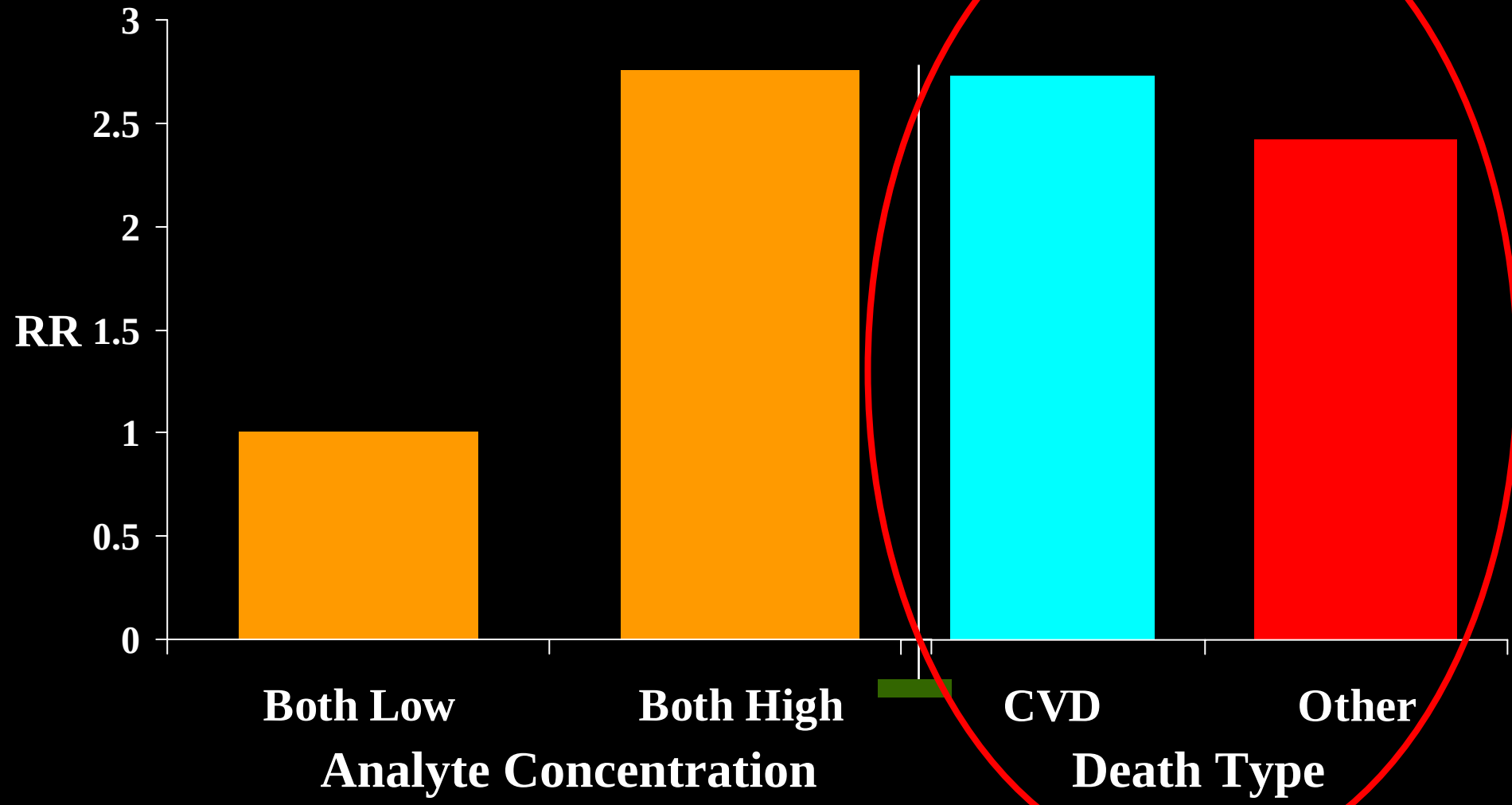
# CRP, IL-6 and Mortality: Iowa 65+ Rural Health Study




Harris et al *Am J Med* 1999;106:506

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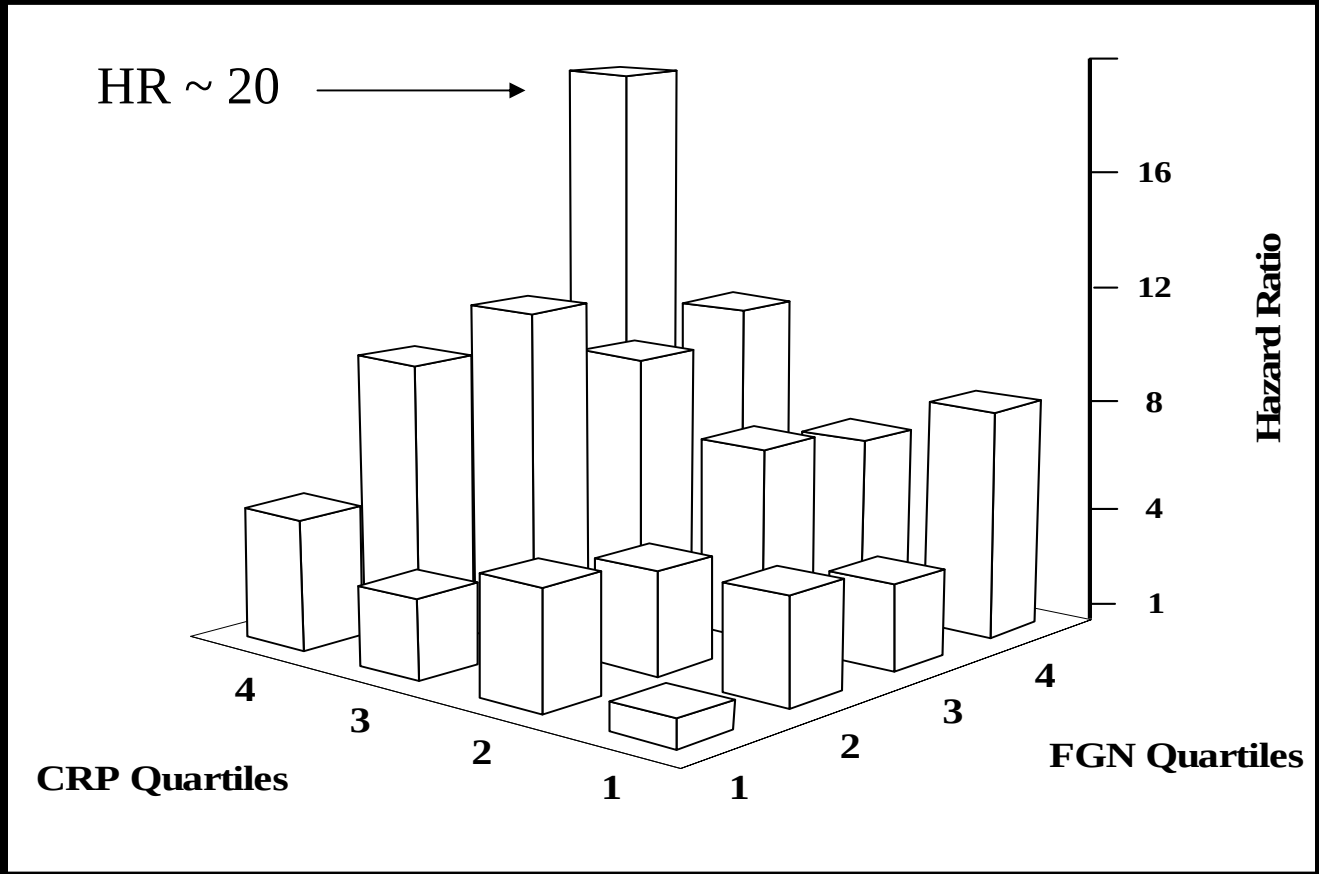
# Fibrinogen and CRP are independent biomarkers of early mortality in elderly men

Cardiovascular Health Study: N ~2500 men >65 years at baseline
The outcome is CVD mortality within 3 years of baseline




Jenny N, et al., manuscript submitted

# The Role of Inflammation in Cronic Diseases & Aging

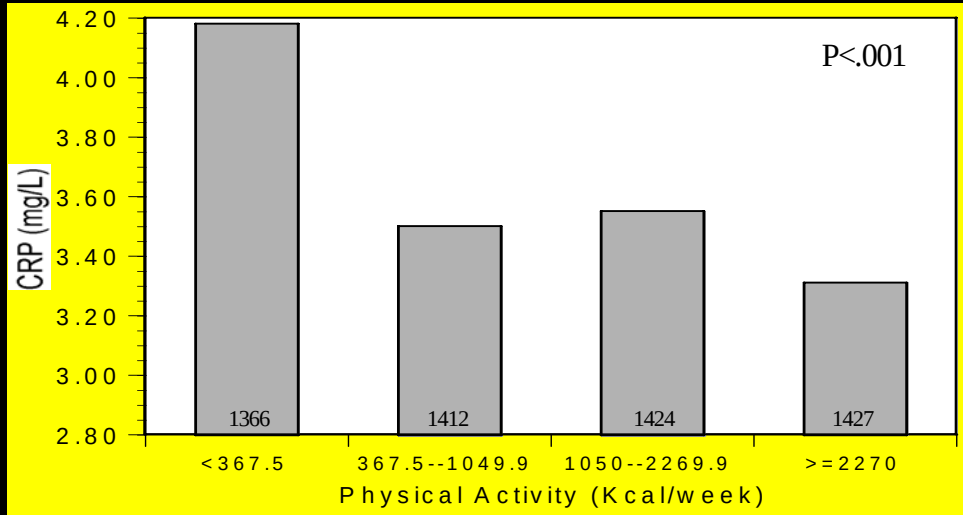
Other outcomes associated with higher inflammation markers:

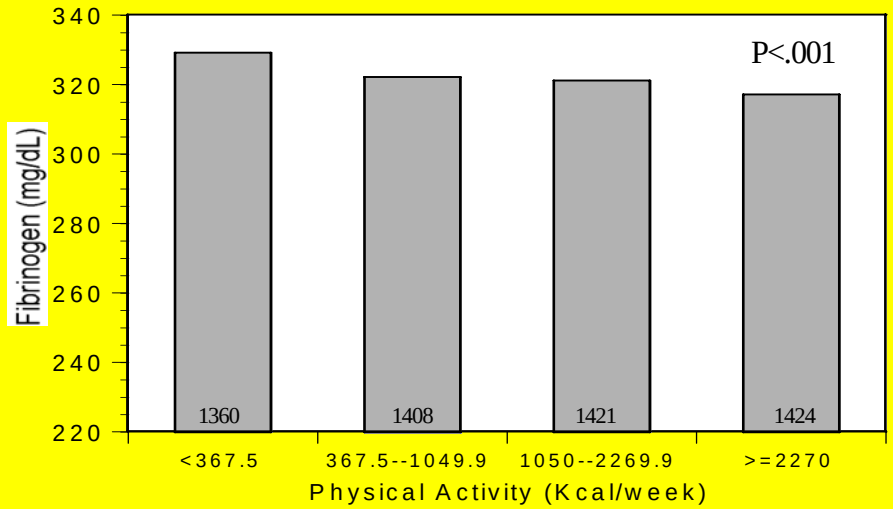
All cause mortality
Type 2 diabetes
CHF
Some cancers (short “lead times”)
Cognitive decline
Osteoporosis
Sarcopenia & Frailty

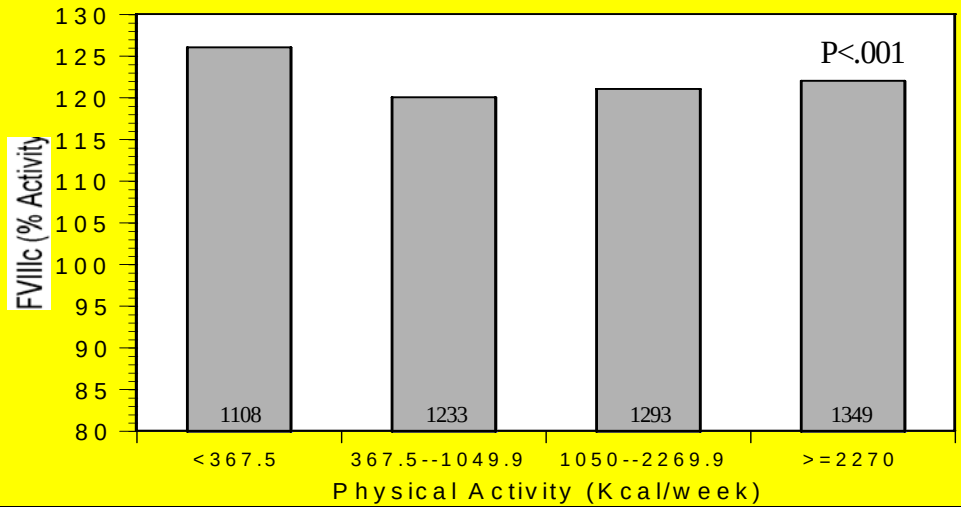
All chronic diseases of old age (?)

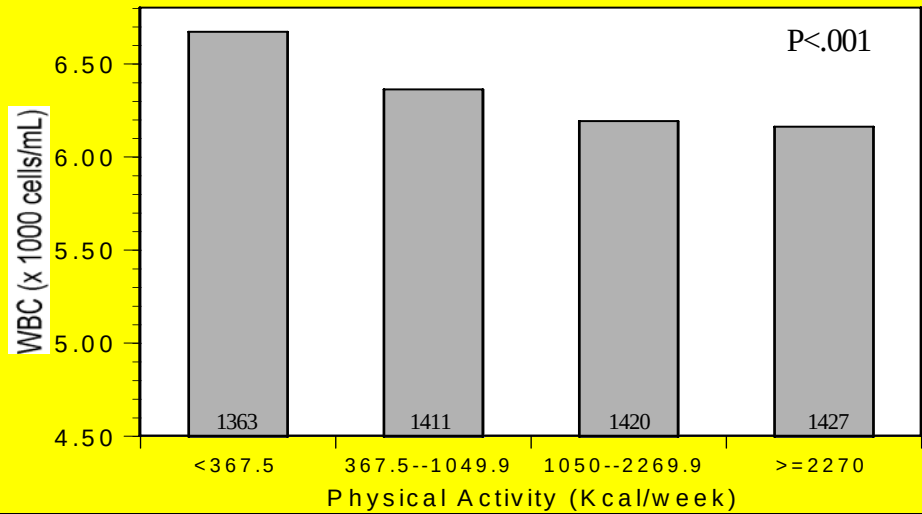
# The Roles of Exercise and Weight Loss

# Association of Activity with Markers of Inflammation




Geffken, et al., *Am J Epidemiol*. 2001;153:242-250

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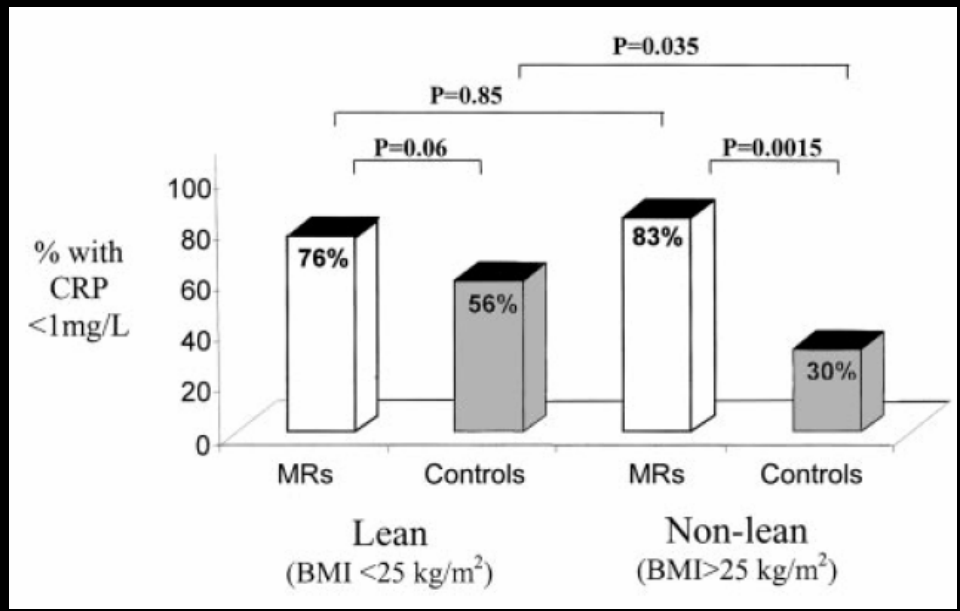
# Association of Activity with Markers of Inflammation

Marathon running shows the two sides of strenuous exercise: acute vs long-term effects

**TABLE 3. Premarathon and Postmarathon Concentrations of sICAM-1, E-selectin, CRP, and Leptin**

| | Premarathon | Postmarathon | *P* |
|---|---|---|---|
| sICAM-1, ng/mL | 203.7 (55.7) | 192.4 (46.6) | NS |
| E-selectin, ng/mL | 60.2 (28.4) | 60.1 (28.0) | NS |
| CRP, mg/L | 0.3 (0.2–0.7) | 1.8 (1.0–3.4) | <0.0001 |
| Leptin, ng/mL | 1.7 (1.2–2.2) | 0.9 (0.5–1.3) | <0.0001 |

Data are mean (SD) or median (interquartile range).




Tomaszewski et al., *Arterioscler Thromb Vasc Biol*. 2003

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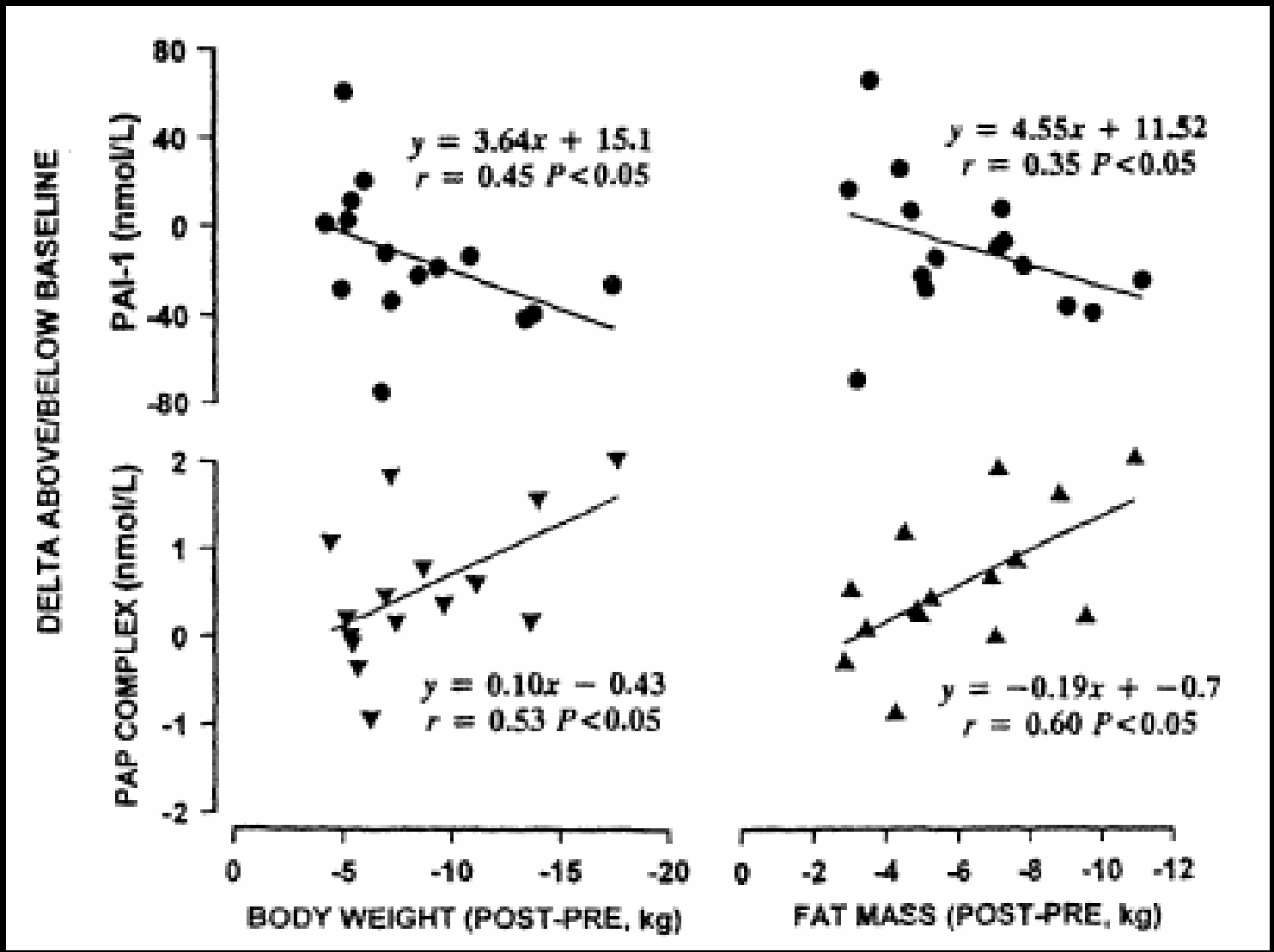
# Association of Weight Loss with Markers of Inflammation




Calles-Escandon J, Tracy R, et al. *Amer. J. Clin. Nutr.* 1996;64:7-11

# Association of Weight Loss with Markers of Inflammation

12-week caloric restriction; ave weight loss 7.9 kg

**TABLE 2. Biochemical Characteristics Before and After Weight Loss**

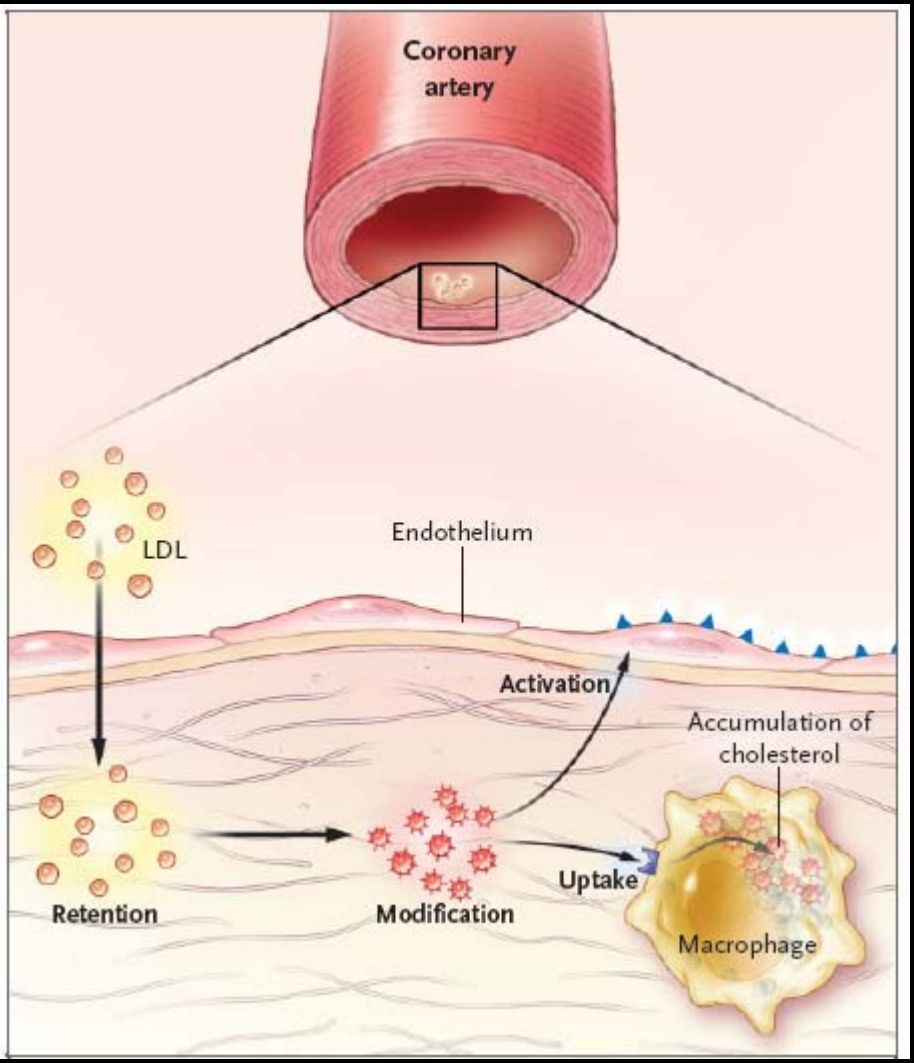
| | Week 0 | Week 12 |
|---|---|---|
| Total cholesterol, mmol/L | 5.69±0.08 | 5.11±0.09* |
| LDL-C, mmol/L | 3.79±0.08 | 3.38±0.08* |
| HDL-C, mmol/L | 1.15±0.03 | 1.08±0.03* |
| Triglyceride, mmol/L | 1.67±0.06 | 1.44±0.06* |
| Glucose, mmol/L | 4.90±0.07 | 4.79±0.05 |
| CRP, mg/L | 5.56±0.36 | 4.12±0.36* |

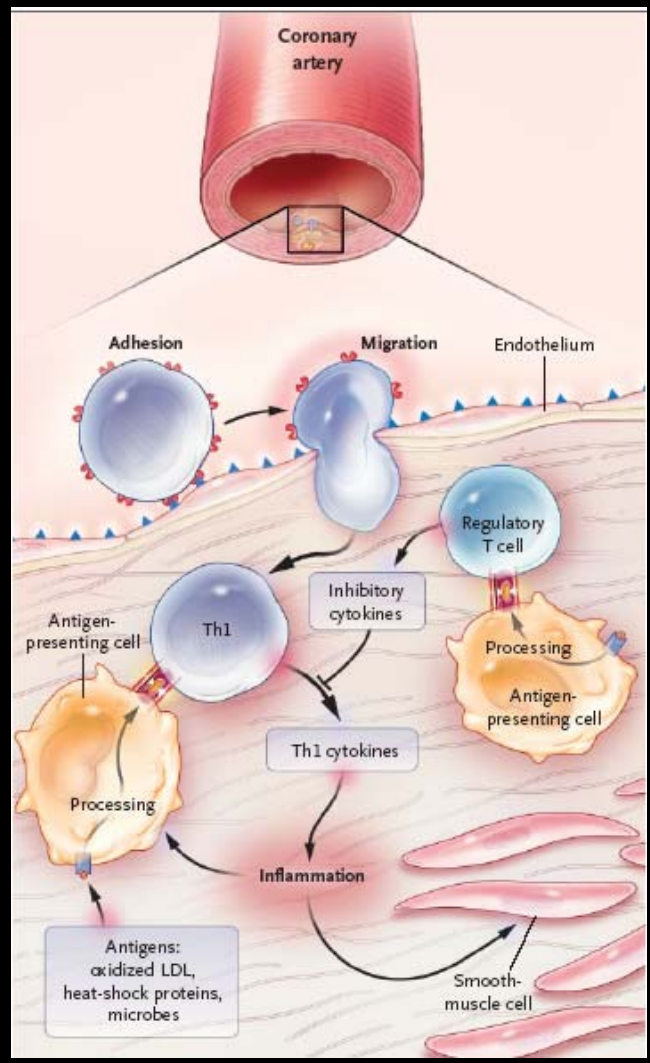
Values are mean±SEM.
*$P<0.001$ vs week 0.

Heilbronn LK. *Arterioscler Thromb Vasc Biol*. 2001;21:968-70

# The Question of the Causal Pathway

# Innate and Adaptive Immunity in Human Atherosclerosis




**Plus other components of the Innate Immune System such as:**

**- Complement**
**- Pentraxins**
*** CRP**
*** SAP**
*** PTX-3**
**-MØ TF→ IIa**

**Hansson G** *N Engl J Med* 2005;352:1685-95

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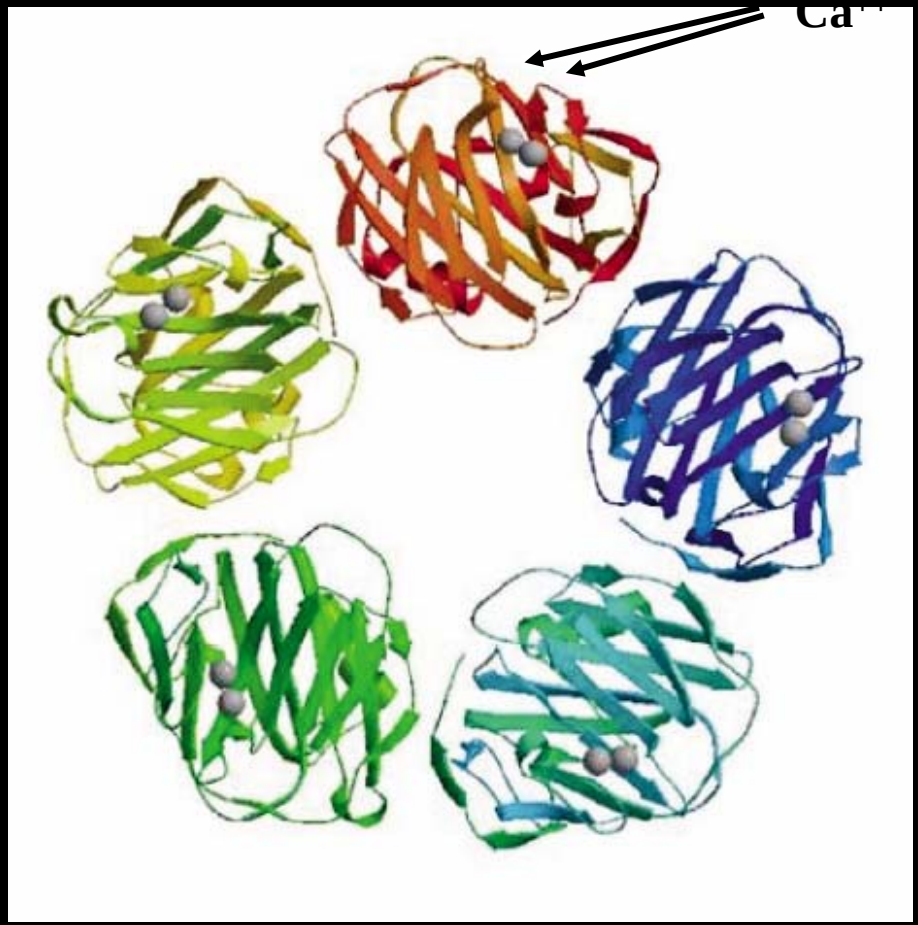
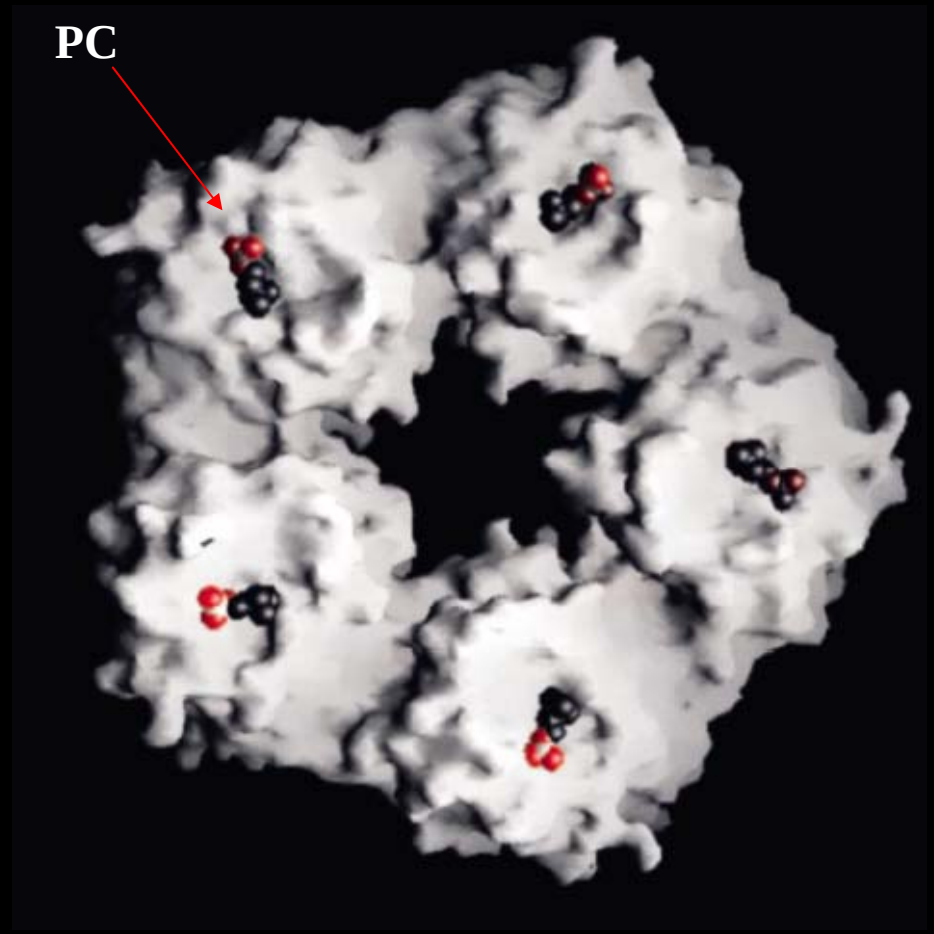
# …then the Innate Immune System will respond…

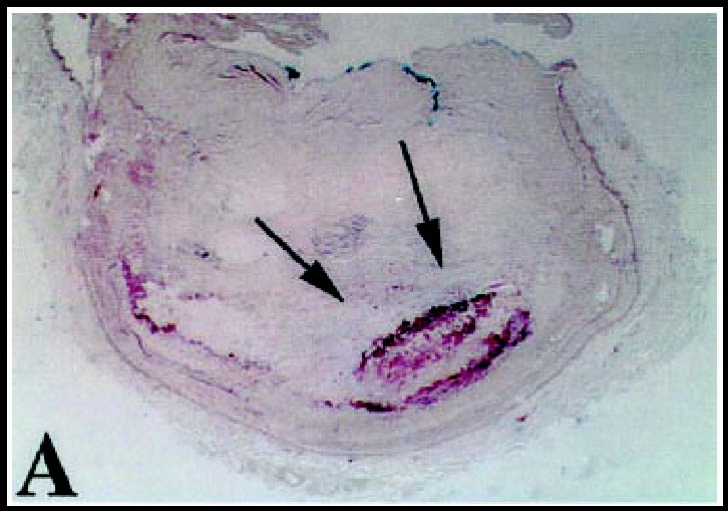
Focus on CRP, an Acute Phase Protein & one of two major human Pentraxins

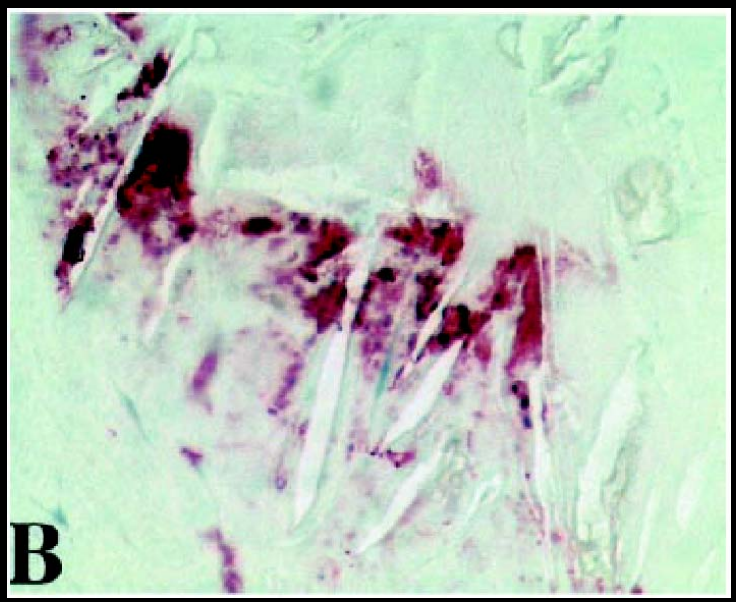


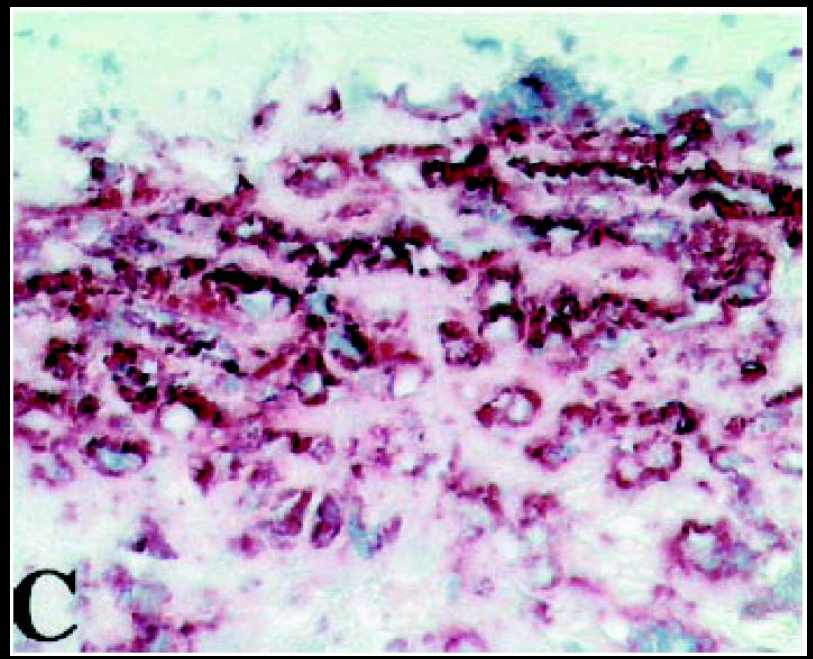




Mr = 155,000/subunit  Binding been shown to: PC and many other PLs; native & modified LDL and other LPs; damaged and apoptotic cells

# CRP Binds Subendothelial Lipids in Atherosclerotic Plaque




A, Lower magnification of an epicardial coronary artery with near total occlusion demonstrates diffuse CRP staining of lipid core area (arrow). B, Higher magnification of this area shows CRP staining adjacent to cholesterol clefts. C, Localization in the cytoplasm of macrophages at the rim of the lipid core.

Burke AP, Tracy RP et al. *Circulation*. 2002;105:2019-23

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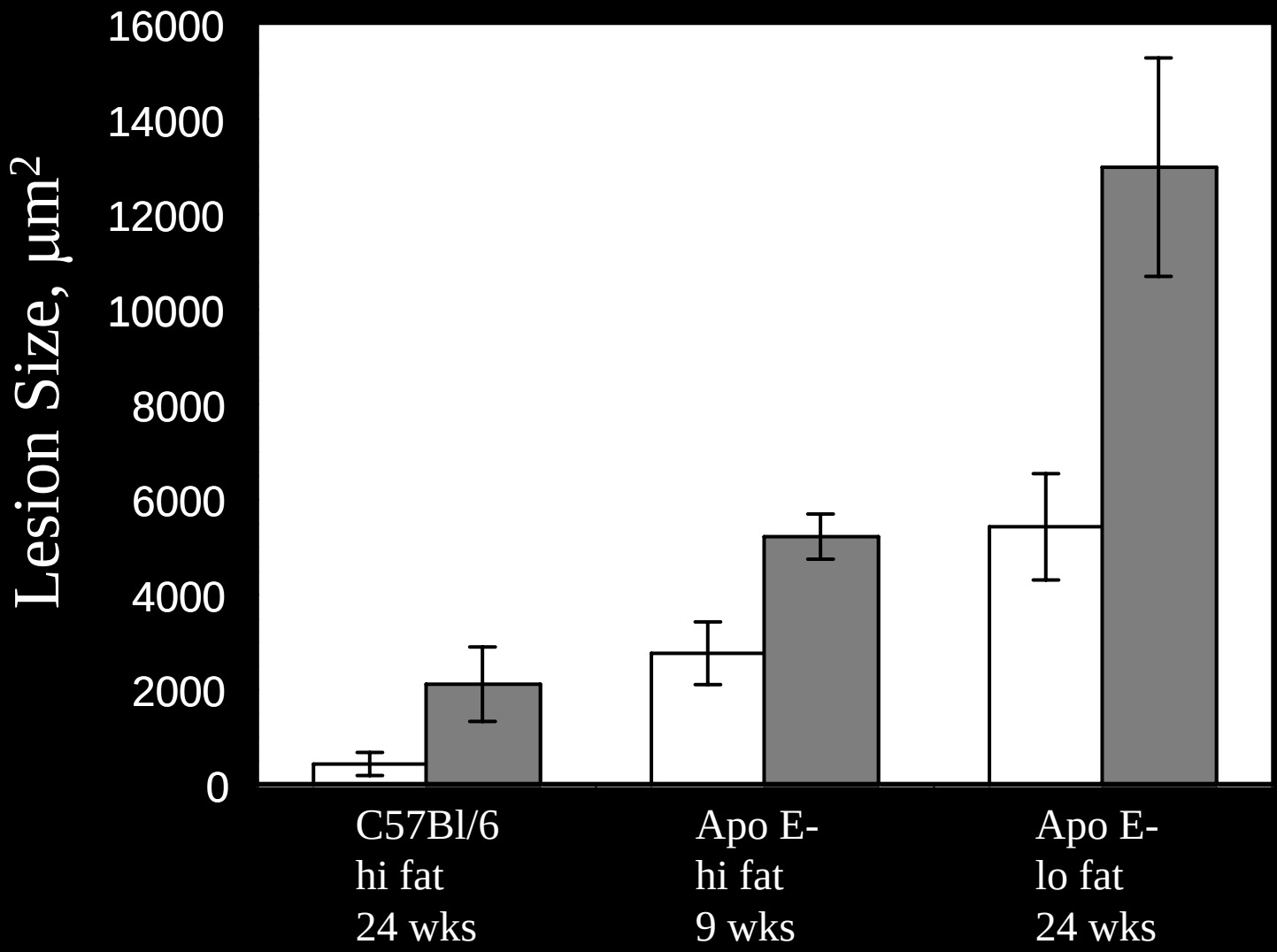
# The Adaptive Immune Response

- Epidemiological studies show that blood levels of IL-6 (and IL-6-responsive proteins) can predict future cardiovascular events
- While IL-6 is the major regulator of acute phase response, it also promotes lymphocyte proliferation and differentiation
- Substantial numbers of T cells and macrophage are present in atheromas and may contribute to atherogenesis
- We hypothesized that IL-6 administration would increase early atherosclerosis in mice

# Effect of Weekly Injections of IL-6




Huber SA, et al. *Arterioscler Thromb Vasc Biol*. 1999;19:2364-7

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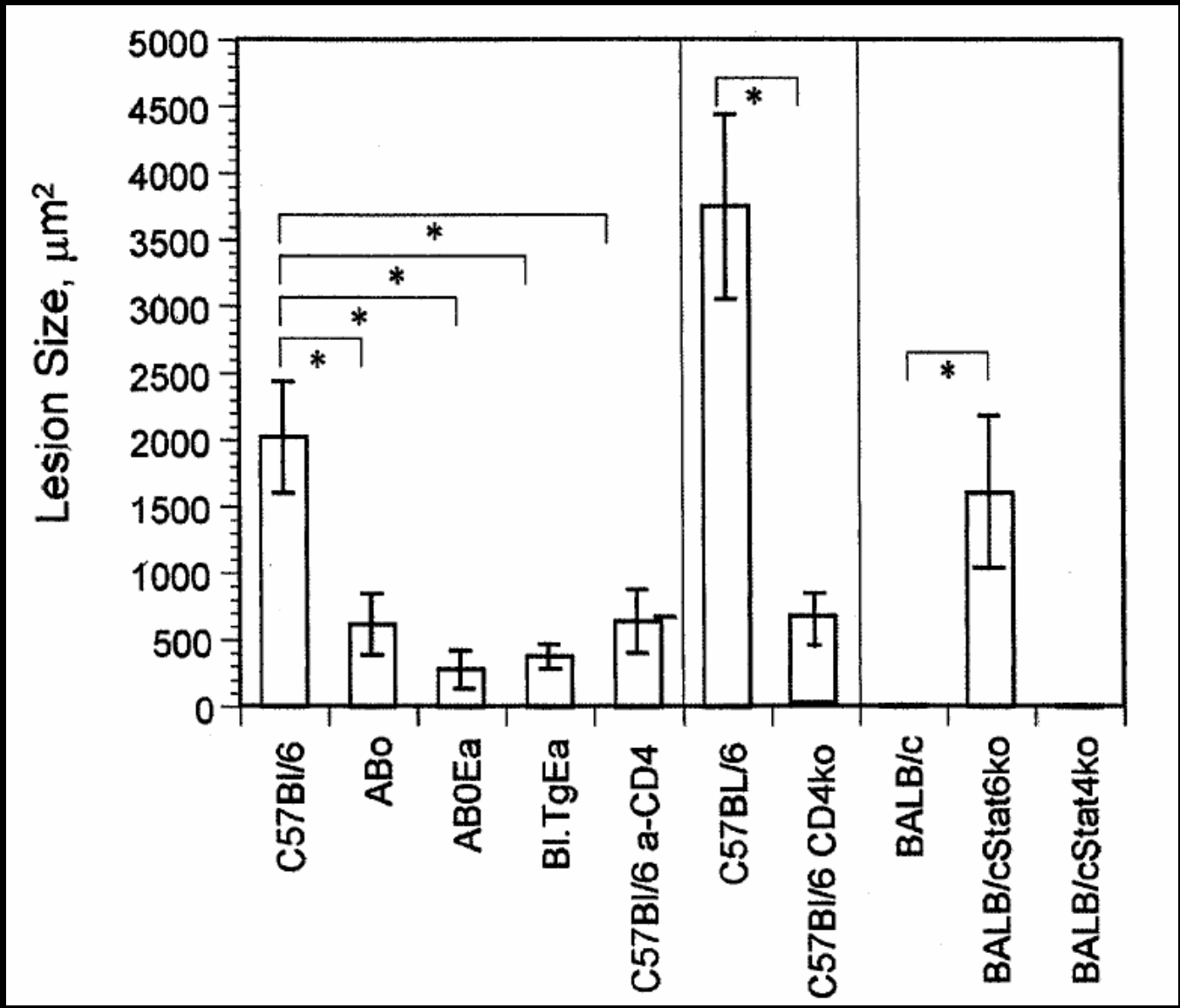
# IL-6 is a major cytokine regulator of T Cell differentiation

- Type 1 T Helper (Th 1) cells primarily augment cell-mediated immunity and produce IFNγ as their major cytokine
- Type 2 T Helper (Th 2) cells primarily augment Ig-mediated immunity and produce IL-4 as their major cytokine
- IFNγ is known to activate macrophages and may play an important role in lipid uptake and foam cell development

# Th1 Phenotype Corresponds to Increased Lesion Size




**Each bar represents average of 4-10 mice and 4 10u sections from the proximal aortic arch.**

*** = P ≤ 0.05**

Huber et al.., Circ, 103:2610, 2001

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# rIL-4 Suppresses Th1 Cell Development and Atherosclerosis in C57Bl/6 Mice

| | PBS | rIL-4 |
|---|---|---|
| Aortic Lesion ($\mu m^2$) | 1996 ±518 | 197 ±102* |
| % Th1 Cells | 32.6 ± 1.8 | 2.5 ± 0.2* |
| % Th2 Cells | 2.3 ± 0.3 | 3.5 ± 0.2* |

* $p<0.01$ compared to PBS

Huber, Tracy et al., unpublished

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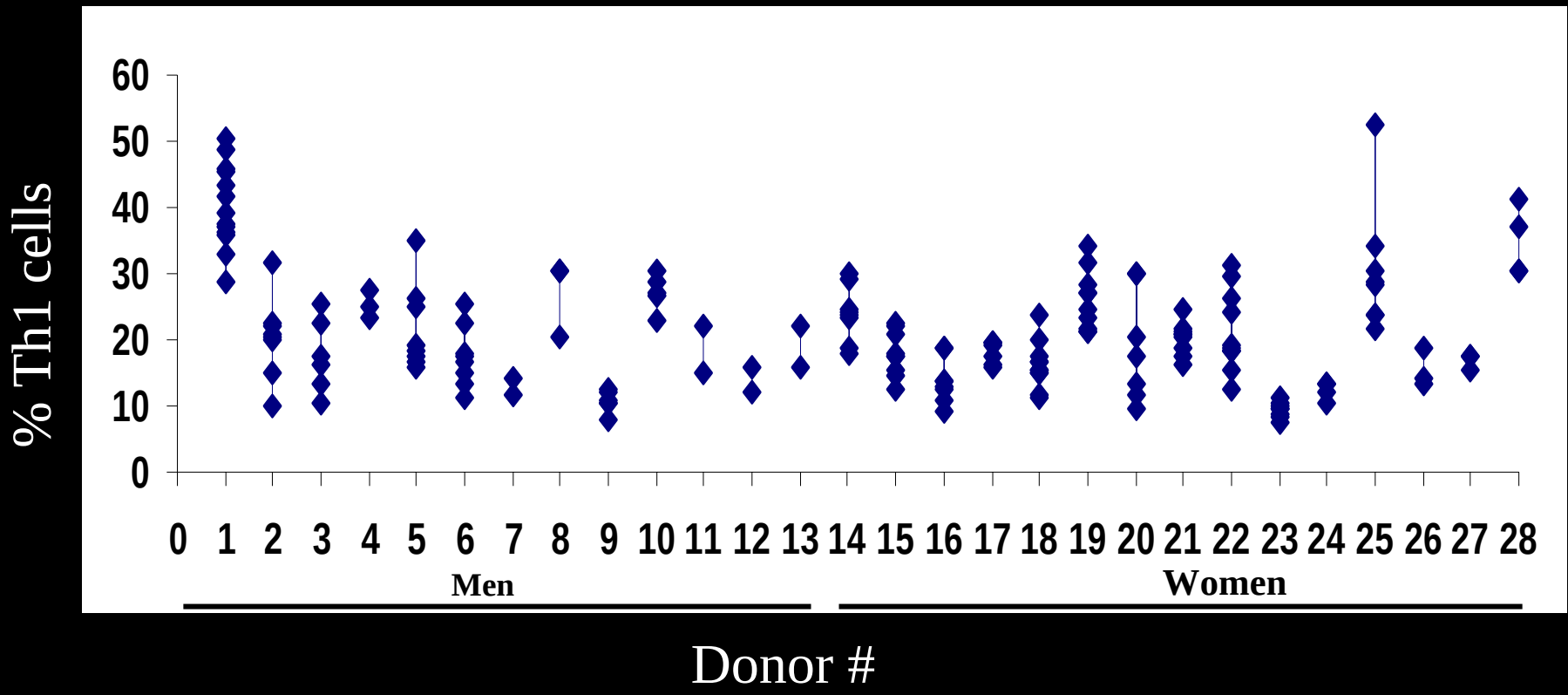
# Population Science and Cell Biology

Can we study these cellular phenotypes in human populations?

## Cellular Epidemiology

Main Hypothesis: %Th1 cells will be possitively associated with atherosclerosis

# % Th1 cells in Healthy Men & Women




**13 Men & 15 Women**
**Average 6 time points /person**
**Avg: 21 +/- 0.67 % Min 7.5% Max 53%**

# Associations Among Secreted Cytokine

## Preliminary Data in establishing cytokine profiles

| | | ccs tnfa | ccs ifng | ccs gmcsf | ccs il10 | ccs il6 | ccs il4 | ccs il2 |
|---|---|---|---|---|---|---|---|---|
| ccs tnfa | Pearson Cor. | | 0.510035397 | 0.8290 | 0.5905 | 0.7599 | 0.5733 | 0.4873 |
| | Sig. (2-tailed) | | 0.0624 | 0.0002 | 0.0262 | 0.0016 | 0.0321 | 0.1081 |
| | N | | 14 | 14 | 14 | 14 | 14 | 12 |
| ccs ifng | Pearson Cor. | | | 0.4059 | 0.5590 | 0.2818 | 0.3003 | 0.2278 |
| | Sig. (2-tailed) | | | 0.1499 | 0.0377 | 0.3290 | 0.2969 | 0.4765 |
| | N | | | 14 | 14 | 14 | 14 | 12 |
| ccs gmcsf | Pearson Cor. | | | | 0.5807 | 0.7369 | 0.5156 | 0.7107 |
| | Sig. (2-tailed) | | | | 0.0294 | 0.0026 | 0.0591 | 0.0096 |
| | N | | | | 14 | 14 | 14 | 12 |
| ccs il10 | Pearson Cor. | | | | | 0.4054 | 0.5890 | 0.5260 |
| | Sig. (2-tailed) | | | | | 0.1504 | 0.0267 | 0.0790 |
| | N | | | | | 14 | 14 | 12 |
| ccs il6 | Pearson Cor. | | | | | | 0.1205 | 0.4926 |
| | Sig. (2-tailed) | | | | | | 0.6817 | 0.1038 |
| | N | | | | | | 14 | 12 |
| ccs il4 | Pearson Cor. | | | | | | | 0.6588 |
| | Sig. (2-tailed) | | | | | | | 0.0198 |
| | N | | | | | | | 12 |

**P <0.15**

**P <0.05**

**P <0.01**

**There are strong associations among secreted cytokines**

# What are we measuring…..

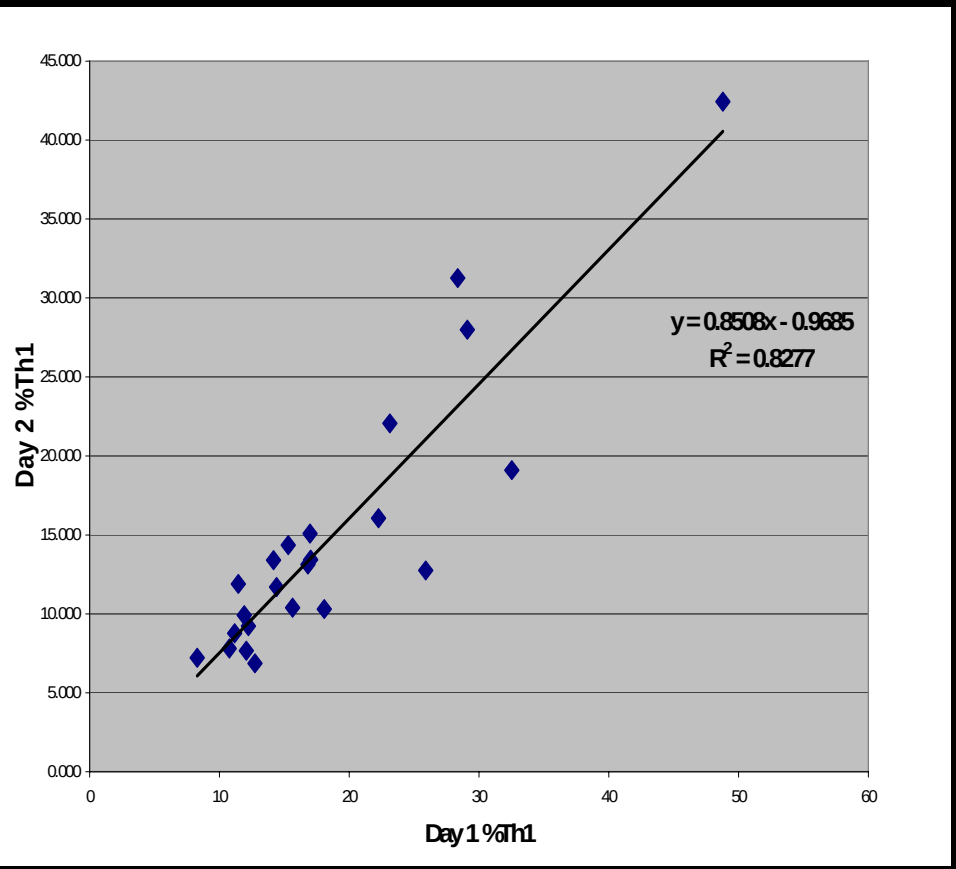
In the MESA 1000 (group 3):

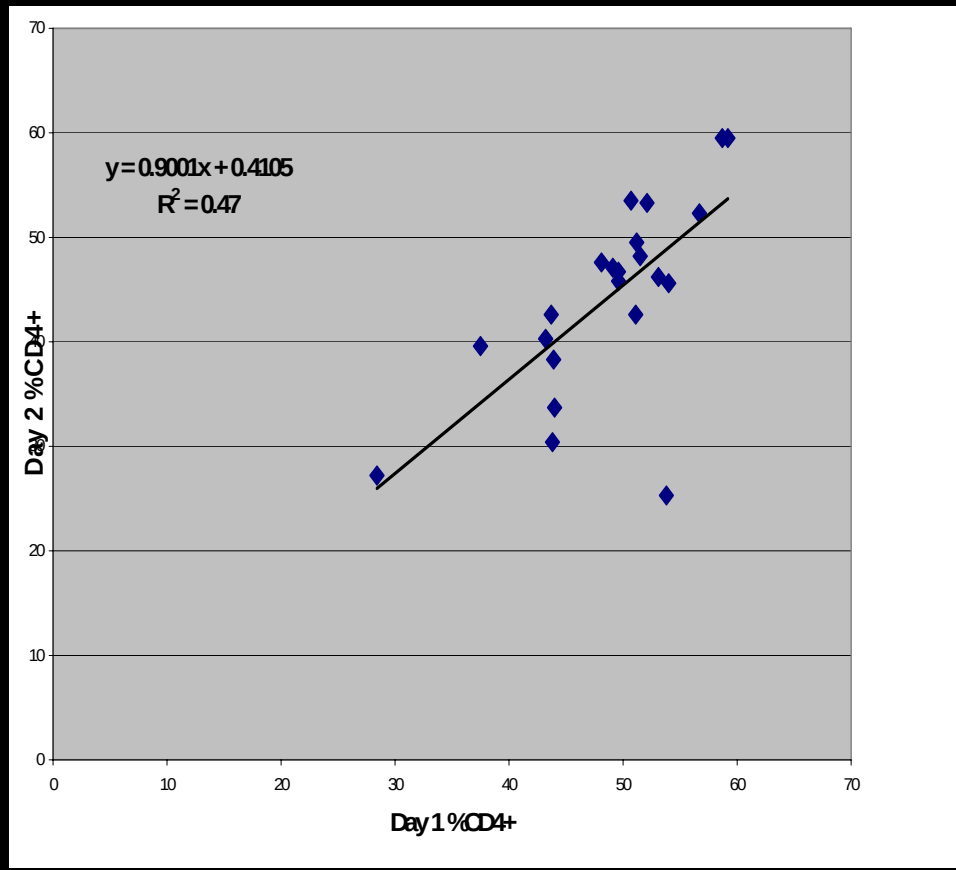
- Innate Immunity:
  - MØ Tissue Factor in response to LPS
    - (CRP, CRP/LDL complex)
  - Gamma-delta T cells
  - NK T cells
  - Plasma biomarkers such as CRP, SAP, PTX-3
- Adaptive Immunity:
  - %Th1 cells, %Th2 cells
  - Secreted IFN-g, IL-4
    - (Secreted cytokine profiles)
  - T memory & T naïve cells
  - (T Regulatory Cells)
- Endothelial Health:
  - Endothelial Progenitor Cells
- Associations: gender, ethnicity, age; risk factors; athero measures
- Outcomes: CVD events, strokes, mortality

# How to do QA/QC in this study?

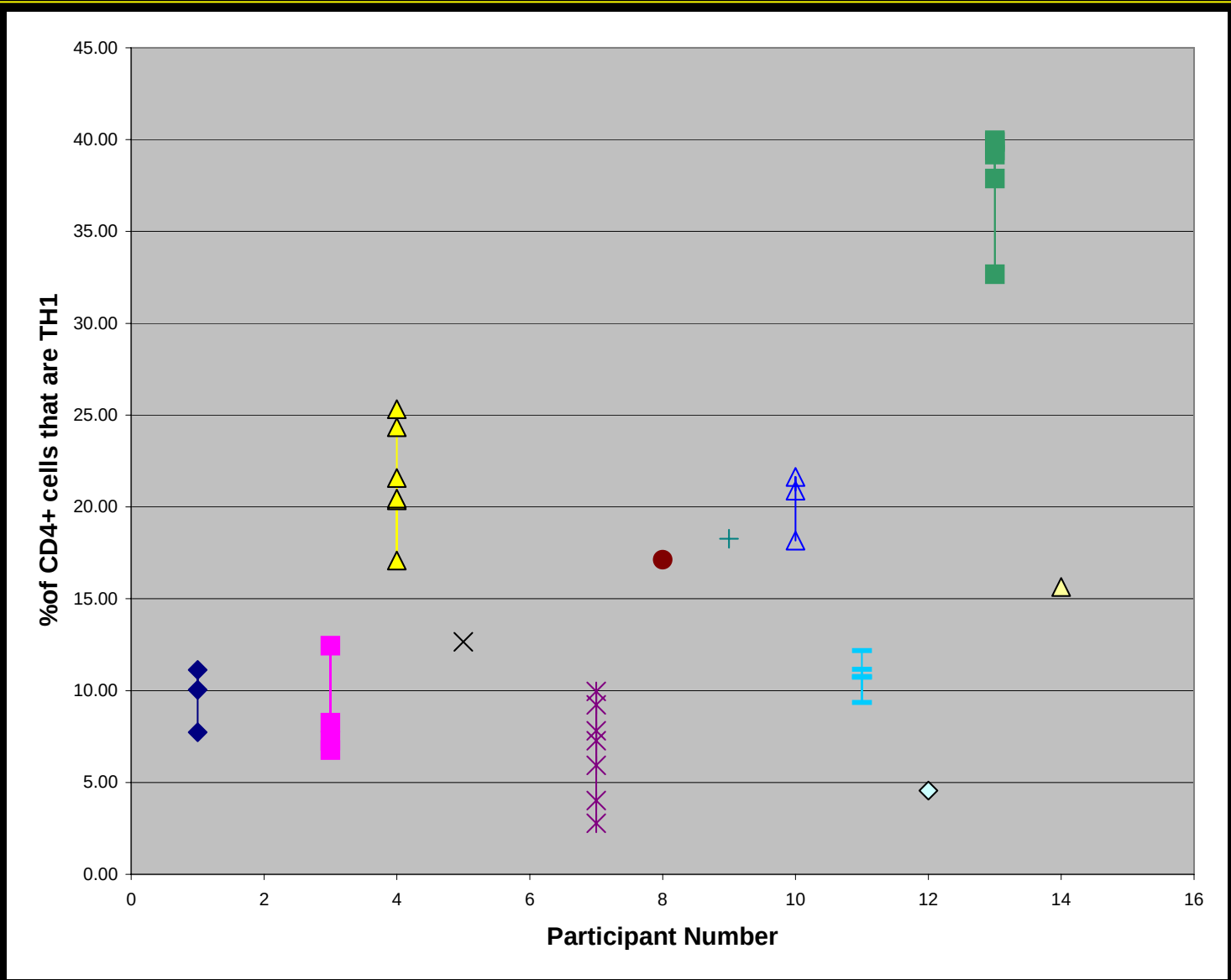
- We've designed a four part approach:
  - First, for shipping, we couldn't identify an on-going mechanism; rely on periodic comparison of fresh and shipped samples;
  - Second, there are standard approaches to QA/QC regarding the technical aspects of the flow cytometer: gating, calibration, etc.
  - Third, we recruited a cadre of 16 people; we assay at least 12 of them as a group, 4x/year;
  - Fourth, from these 12, we have identified a subset of 4 people; assay at least one of them each week;

# QA/QC in this study: Fresh vs Shipped Samples

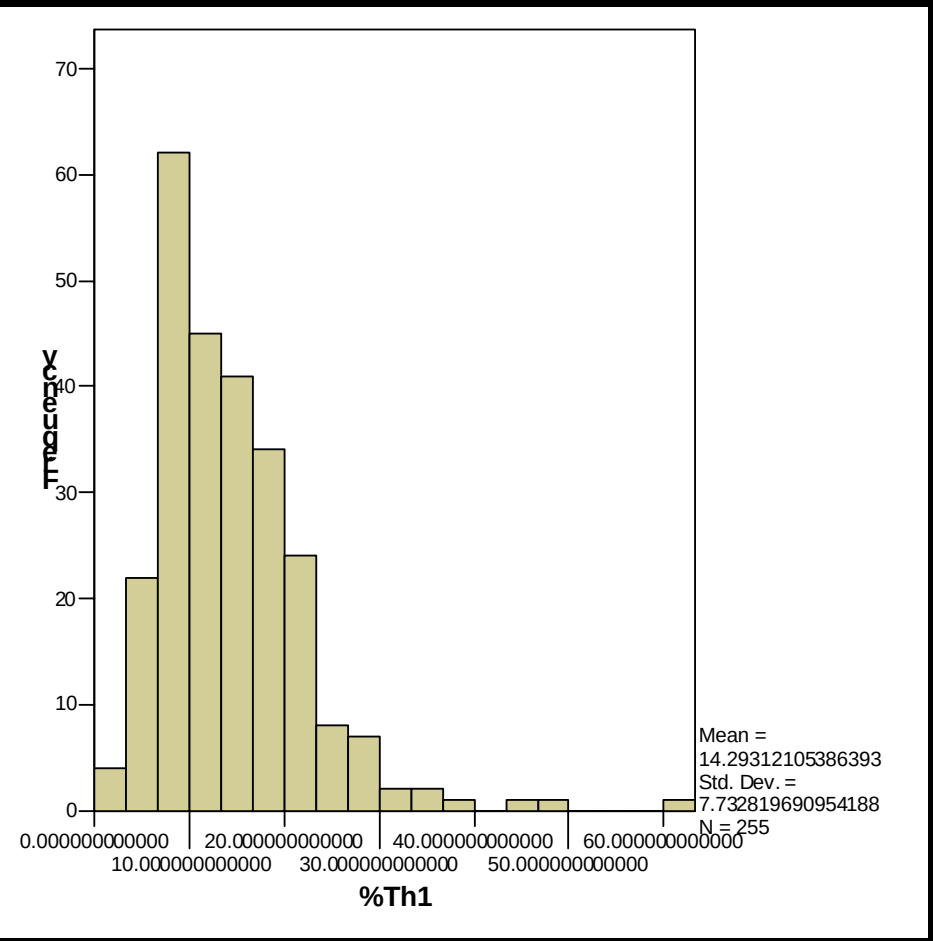


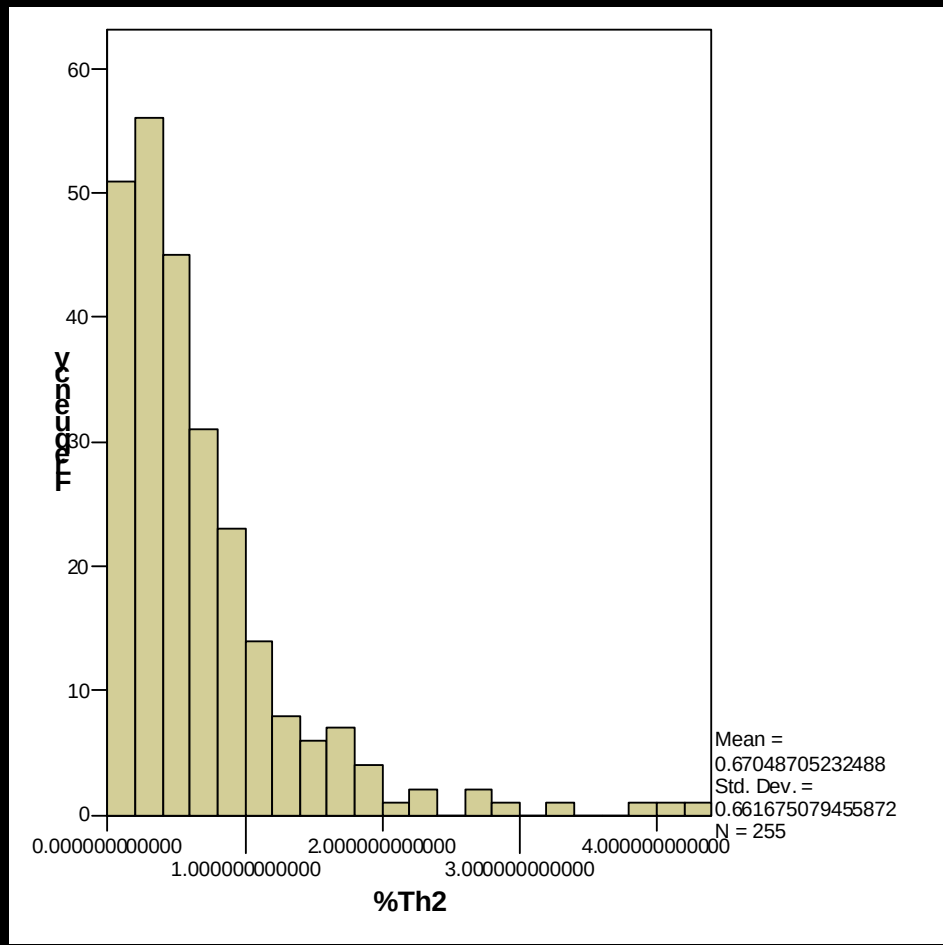



# QA/QC in this study %Th1 cells

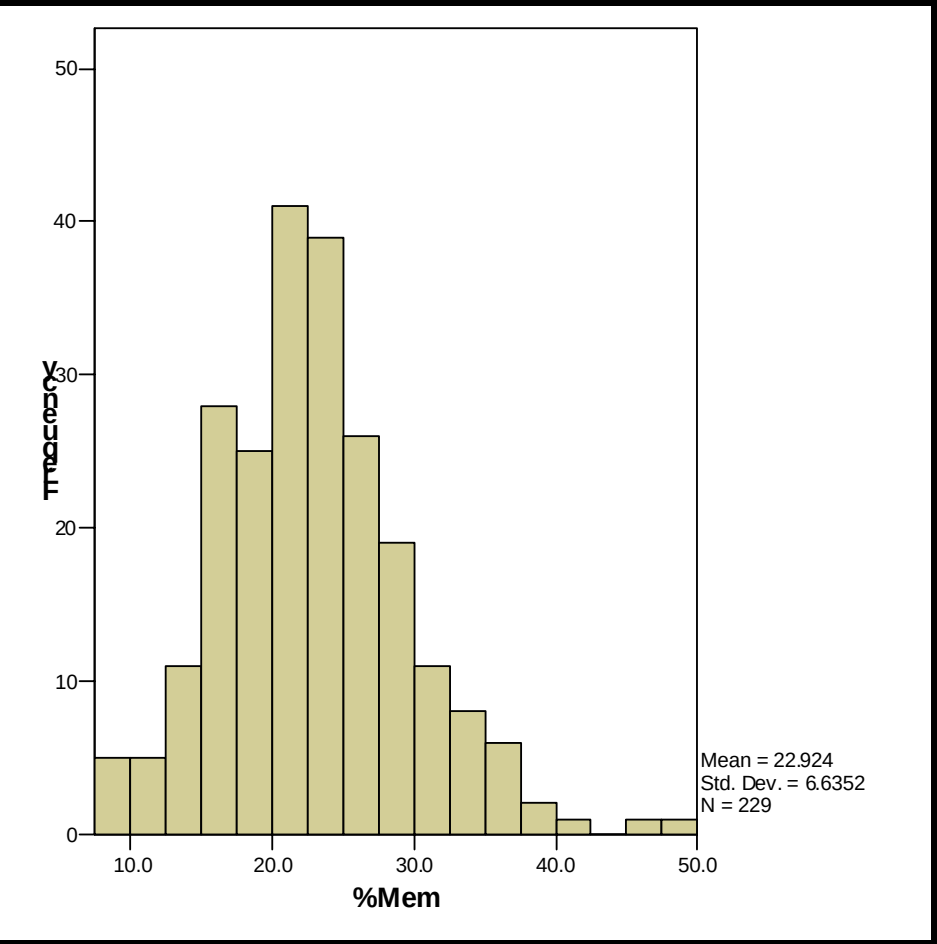


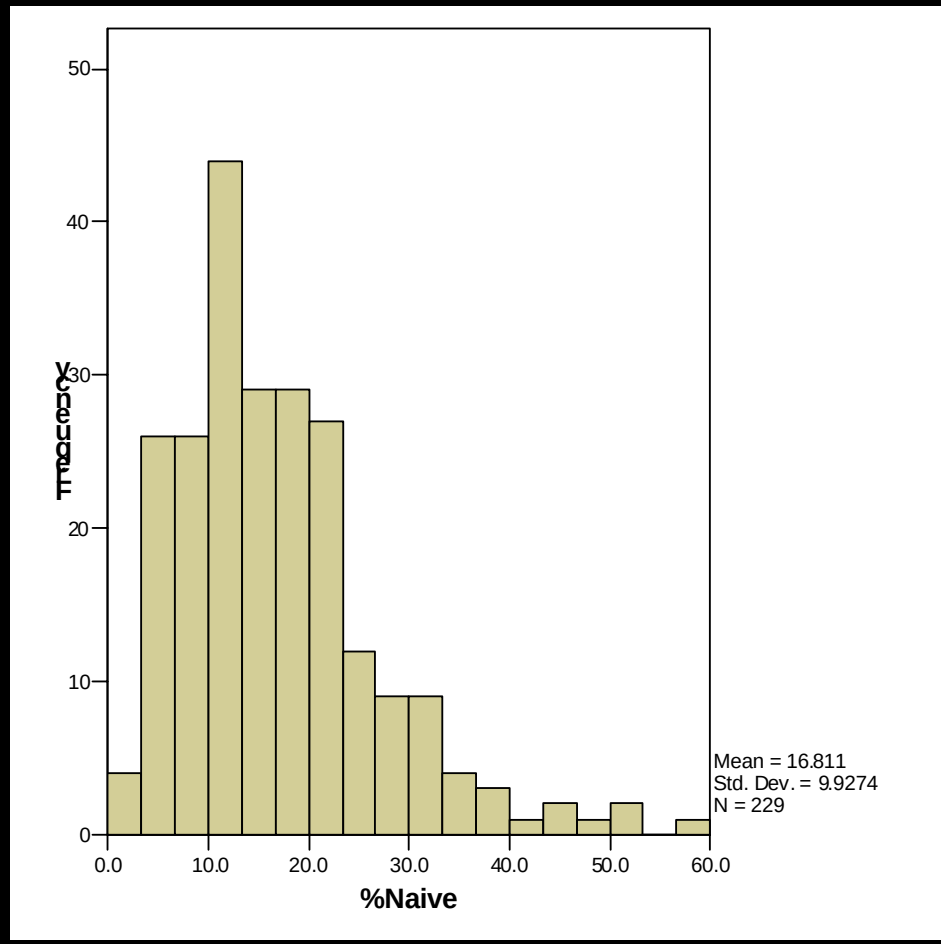
# Distributions: %Th1 cells and %Th2 cells



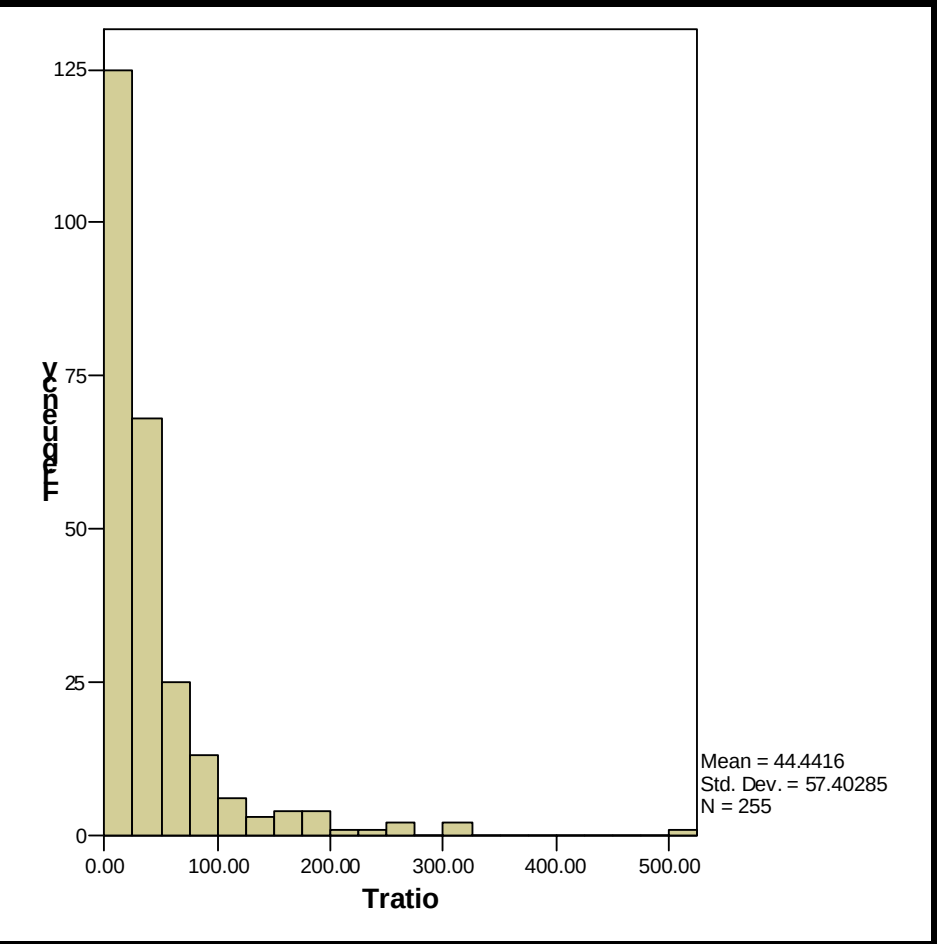



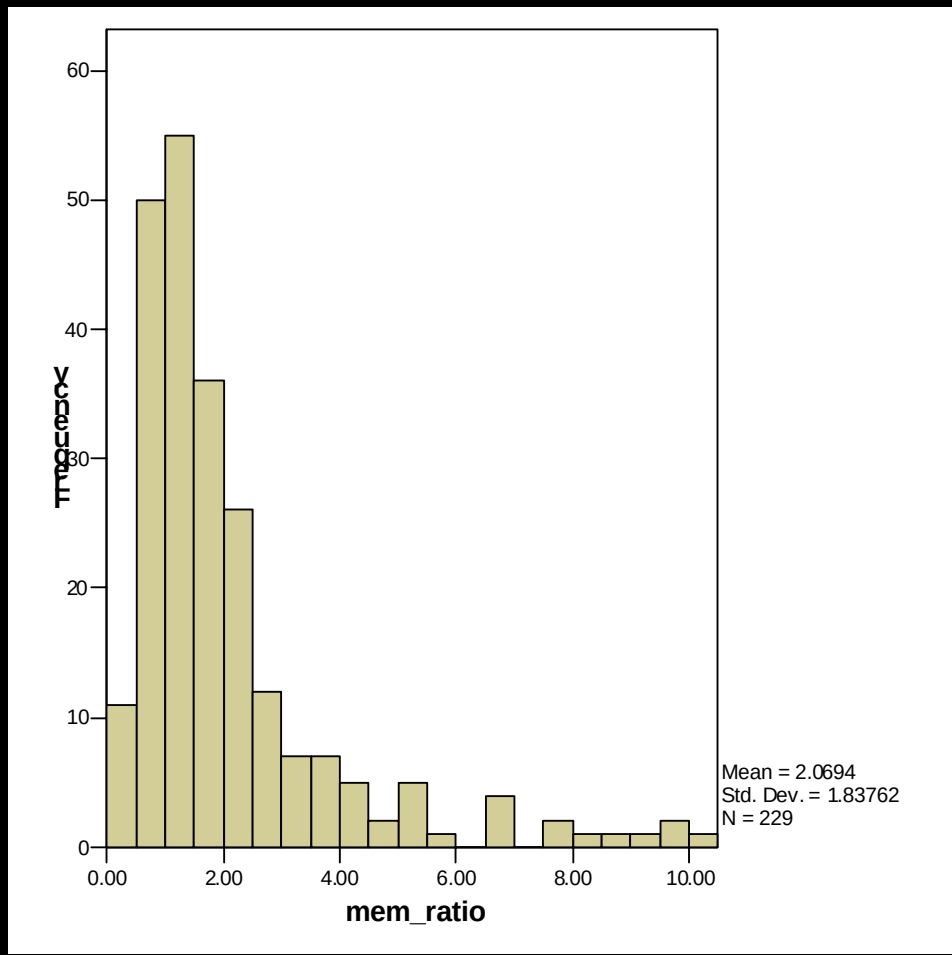
# Distributions: % Memory T cells and % Naïve T Cells



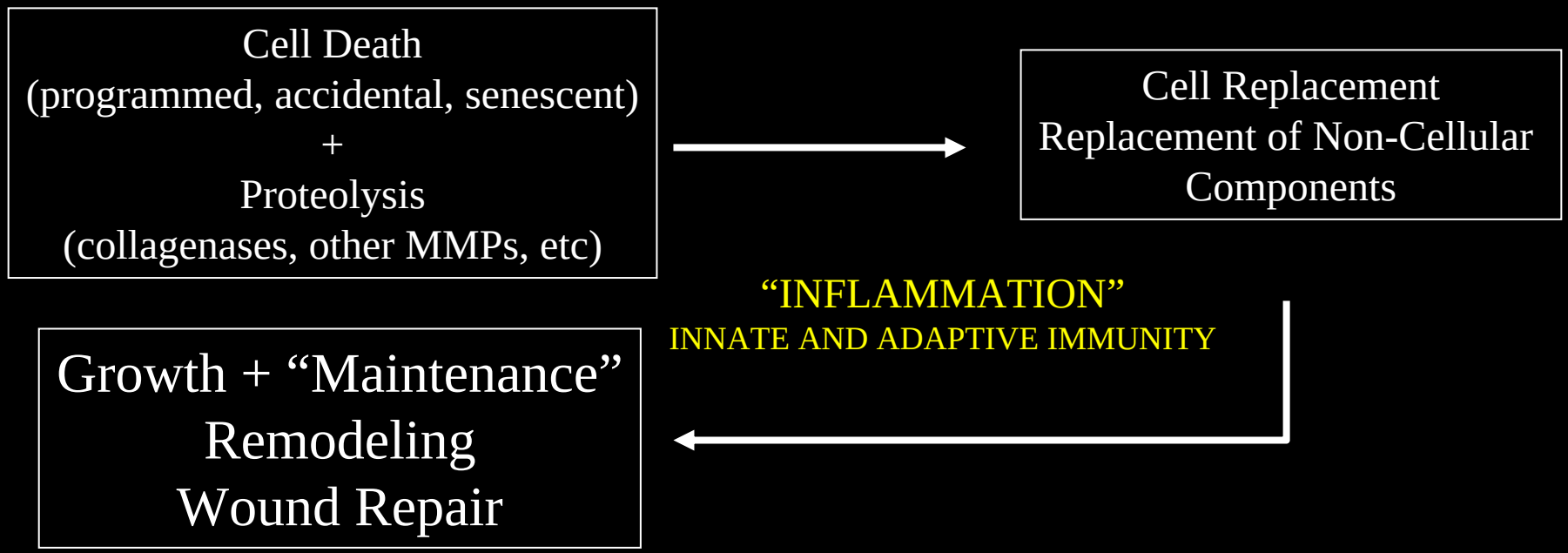



# Distributions: Th1/Th2 ratio and $T_{mem}/T_{naive}$ ratio



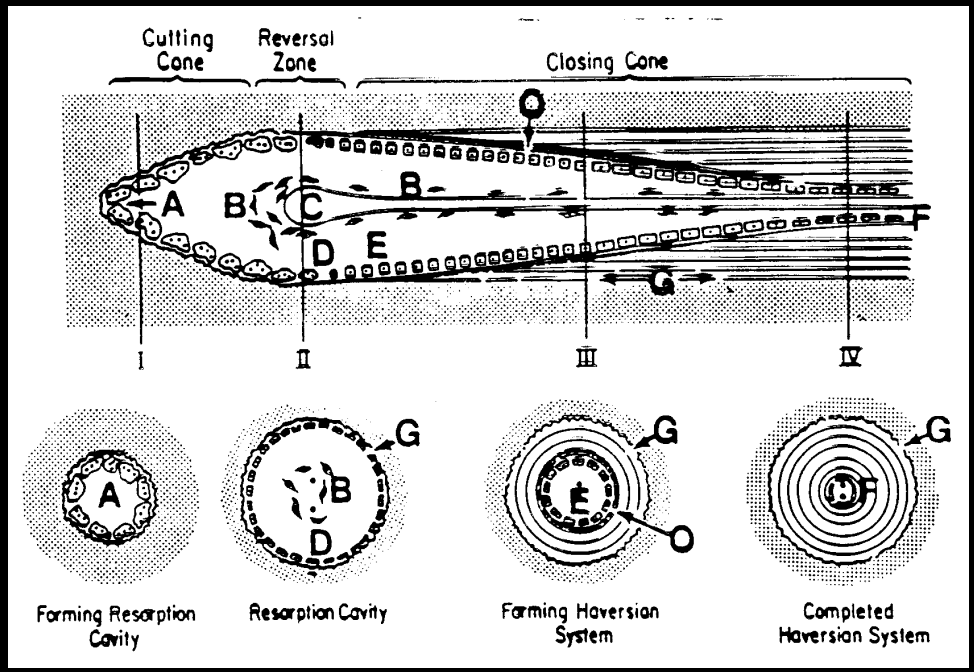



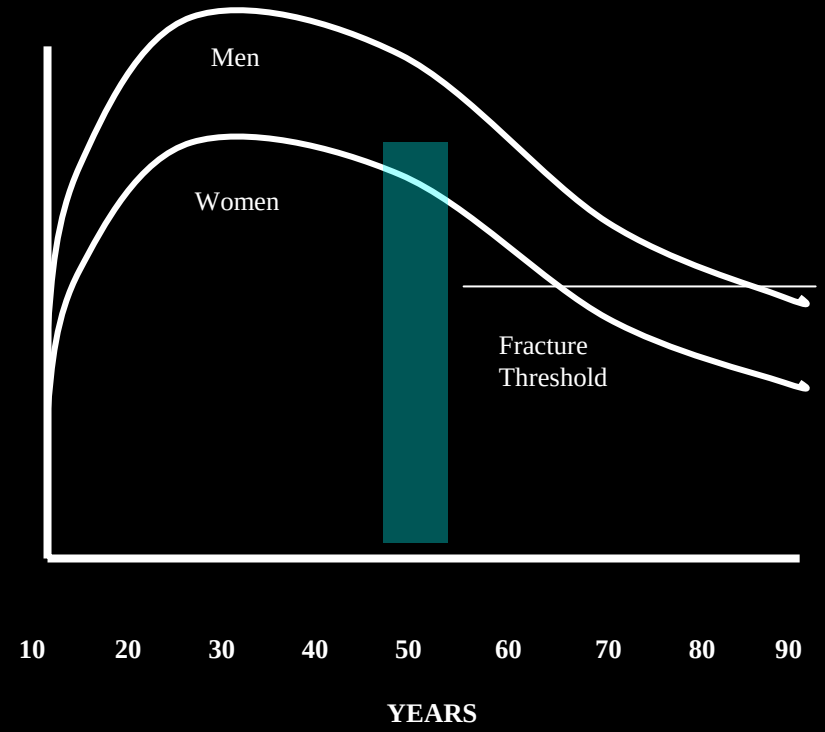
# Hypothesis of Aging: (1) the "background" rate of Aging




Point 1: the "clean up" of this process is "inflammation";
Point 2: early life: net increase in function; later life: net loss of function; why??
It is likely that "inflammation" plays a role through many mechanisms
Point 3: general inflammatory burden may be increased by behaviors (e.g., smoking, sleep disordered breating), environments (e.g., infections), response capacity (e.g., adiposity), and genes (?)

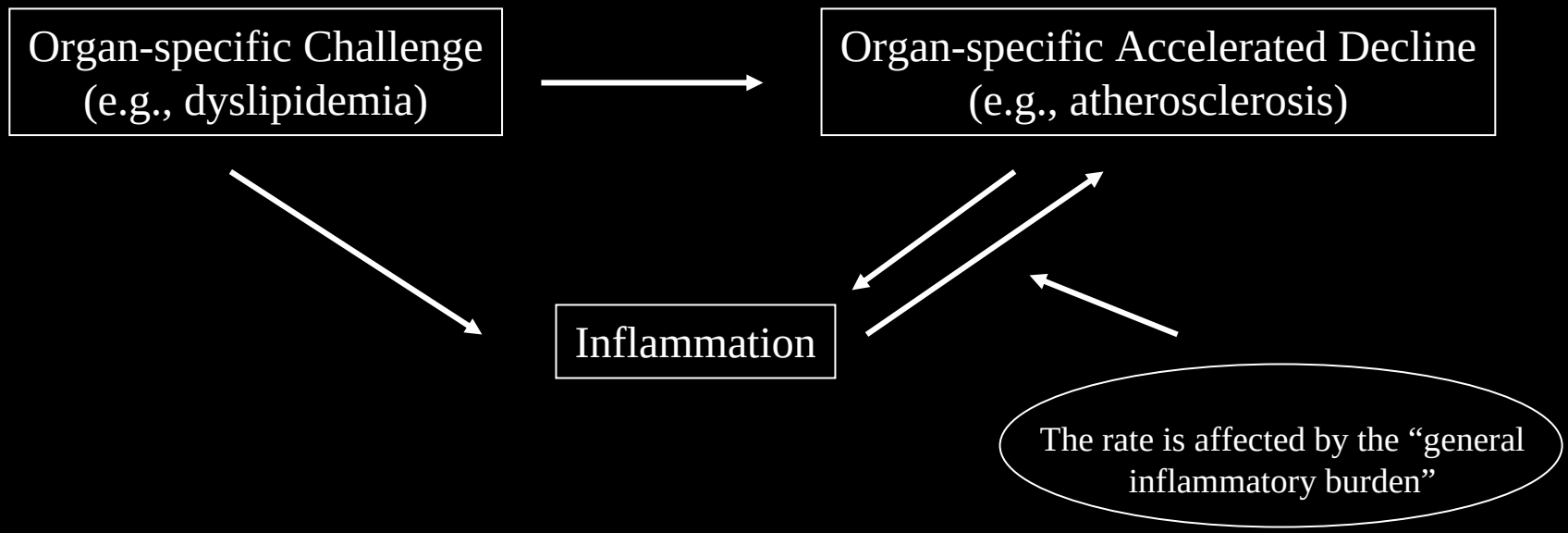
# Bone Remodeling: a Model for a Lifetime of Change?




- In bone remodeling, we resorb and replace ~10% of our skeleton/year;
- Other tissues are slower (brain) or faster (intestinal epithelium);
- Overall rates in all tissues: ??
- This is inflammation too…..

# Hypothesis of Aging: (2) role of specific challenges




1. In providing a necessary "interface" to the environment, "inflammation" can result in damage.
2. The better our responses and/or the more environmental stress to which we respond, the more damage we do.
3. We trade short-term benefit for long-term damage; a good trade from an evolutionary standpoint: Antagonistic Pleiotropy

# Antagonistic Pleiotropy: at the species- and individual-level

- Thrifty Genotype (species-level): genes evolved under conditions of caloric scarcity, might be harmful under conditions of caloric plenty.

  Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? 1962. *Bull World Health Organ*. 1999;77:694-703; discussion 692-3.

- Thrifty Phenotype (individual-level): Metabolic capacity programmed under conditions of caloric scarcity, might be harmful under conditions of caloric plenty.

  Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia*. 1992;35:595-601.

# Conclusion: The "Inflammation Hypothesis" of Chronic Disease

1. In providing a necessary "interface" to the environment, "inflammation" can result in damage.

2. The better our responses and/or the more environmental stress to which we respond, the more damage we do.

3. We trade short-term benefit for long-term damage; a good trade from an evolutionary standpoint

# Why might systems that keep you alive when you're young, contribute to disability when you're old ??